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**Official Organ of The American Phytopathological Society**

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# THE RELATION OF WEATHER TO EPIPHYTOTICS OF LATE BLIGHT ON TOMATOES

A. L. Harrison

(Accepted for publication April 14, 1947)

Attention was called by the writer early in 1946 (4) to an unusual outbreak of late blight of tomato, caused by *Phytophthora infestans* (Mont.) de Bary. The seriousness of the situation was stressed in that publication but the results of the outbreak proved to be far more serious than expected. The disease became extremely destructive in early tomato seed-beds over the west coast area of Florida in January. Hardly an early seed-bed escaped its ravages. From 80 to 90 per cent of the early seed-beds were complete failures and even apparently sound plants pulled from beds slightly affected with late blight were lost on being set in the field during late January.

Although late blight has been reported repeatedly as occurring on tomatoes, most of the reports have dealt with its occurrence on green fruits or on mature vines late in the season. Howitt (5) and more recently Young (10) reported its occurrence on tomato seedlings. In the outbreak of late blight in Florida, tomato seedlings growing in outdoor seed-beds were repeatedly observed to be killed by the late-blight fungus before the first true leaves were formed. In many cases whole beds involving hundreds of thousands of plants were completely destroyed before they were 3 to 4 inches high. No record in the literature has been found where late blight ever appeared so suddenly and so destructively on tomatoes over such a wide area. Before the 1946 spring season was over, it appeared in practically every tomato section in the state of Florida. It was observed first in Hillsborough County on the west coast of Florida on November 26, 1945. There were no commercial acreages of potatoes at the time in this section. It did not cause any appreciable damage to the fall crop except in isolated cases, since it did not become general over the Hillsborough-Manatee County tomato belt until late in December when the fall harvest was practically complete. There was no general killing frost to destroy the vines from the fall crop, so when the seed-beds for the spring crop were started in December and January there was plenty of inoculum to account for the disastrous outbreak of late blight in these tomato seed-beds.

The identity of the fungus responsible for the outbreak has received some attention. Some suggestions were made that it was not *Phytophthora infestans* but some other species such as *P. capsici* Leon. This would seem not to be the case since volunteer pepper plants thrived without any leaf or stem lesions in unsprayed areas of a tomato seed-bed, where tomato plants were completely destroyed. Other pepper plants without any leaf or stem lesions and adjacent to affected tomato plants were observed repeatedly. This is offered as proof that the fungus responsible for the outbreak of late blight was not *P. capsici*.

The first symptoms of late blight on young tomato seedlings appeared as small dark necrotic lesions from 4 to 5 days after periods favorable for inoculation. Sometimes they were on the leaves, sometimes on the stems, and sometimes on the growing points. The lesions rapidly enlarged and soon caused the death of the seedling. If the lesions were on the stem near the ground damping-off occurred, but usually the first lesions were on the leaves or upper portion of the stem. Young seedlings were sometimes killed within 2 or 3 days after the first symptoms were evident. Older plants took longer to succumb to the disease. Large transplants occasionally recovered from late blight when the disease had not progressed far enough to kill all the buds. Such recovery occurred only if the weather changed and became unfavorable for development of the disease. Toughened stem tissues appeared to be more resistant to invasion by *Phytophthora infestans* than were leaf and succulent stem tissues. Stem lesions on young plants were more numerous near the growing tip than they were near the surface of the ground.

A few growers who followed spray recommendations immediately after late blight first appeared in the seed-beds managed to save most of their early plants, but those growers who were caught without proper spray equipment or failed to do a thorough job of spraying lost practically all their early plants.

### INFLUENCE OF WEATHER ON THE SPREAD OF LATE BLIGHT

Following the sudden outbreak of late blight in the tomato seed-beds during the middle of January the weather changed and became unfavorable for the development of late blight and favorable for the growth of tomatoes. Late tomato seed-beds came through in fine shape and the bulk of the spring tomato acreage on the west coast was set with plants from these beds.

Late blight was inactive during February but it reappeared when the weather again became favorable for the development of *Phytophthora infestans* on February 27, and continued to be favorable through March 2. From early in the morning of February 27 until the afternoon of March 2, the relative humidity did not go below 100 per cent except for about seven hours when it ranged from 95 to 100 per cent (Fig. 1). This long duration of high humidity coupled with the fact that the temperatures remained in the 60's or low 70's for the entire time made the conditions ideal for the spread and development of the late blight fungus. New lesions of late blight started to appear on March 2 and by March 4 it was destructive in most unprotected fields of tomatoes. The outbreak of late blight then subsided so that fresh lesions were hard to find by March 10. During the middle of March the weather again became favorable for the spread of late blight, which resulted in another outbreak of late blight about March 19. This phenomenon of late blight becoming destructive and then dying down was repeated six times during the 1946 spring season. There were two periods in January, three in March (the first one of these starting in February), and one in April when late blight was unusually active and destructive.

The outbreak in April was during the height of the harvest season and for this reason probably caused more financial loss than any of the other outbreaks. The farmer's auction market in Palmetto was closed on April

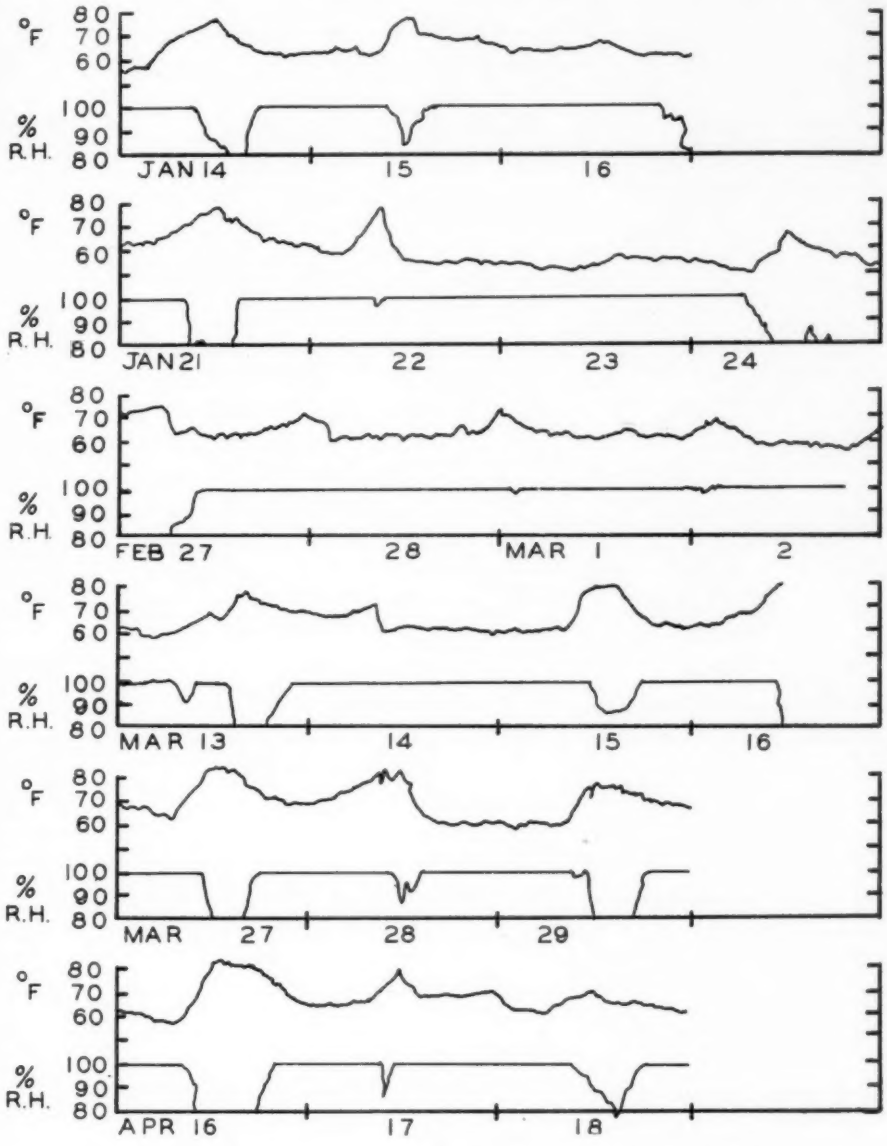


FIG. 1. Temperatures and humidities preceding late blight epiphytotics.

25 and 26 because the tomato fruits were being lost in transit from late blight. Fruits that were apparently sound began to develop late blight 4 to 5 days after they had been picked. The effect of this late spread of late blight was evident in lower prices and slower sales for several days

TABLE 1.—*Relative humidity preceding late blight epiphytotics on tomatoes in the winter of 1946*

| Period | Hours the relative humidity was | | | |
|---|---|---|---|---|
| | 100 per cent | 90–99 per cent | 80–89 per cent | Less than 80 per cent |
| Jan. 14, P.M.–Jan. 16, P.M. | 43 | 4 | 1 | 0 |
| Jan. 21, P.M.–Jan. 24, A.M. | 63 | 2 | 0 | 0 |
| Feb. 27, A.M.–Mar. 2, P.M. | 68 | 7 | 0 | 0 |
| Mar. 13, A.M.–Mar. 16, A.M. | 54 | 3 | 3 | 0 |
| Mar. 27, P.M.–Mar. 29, A.M. | 33 | 2 | 3 | 0 |
| Apr. 16, P.M.–Apr. 18, A.M. | 35 | 1 | 1 | 0 |

before the market closed and continued for several days after it reopened. Growers who had kept their tomatoes sprayed as recommended had little or no loss from late blight on their fruits.

Because there were certain definite periods when late blight was destructive an attempt was made to correlate the weather with destructive outbreaks. Data on the weather that preceded the 6 serious outbreaks of late blight are presented in tables 1 and 2 and in figure 1.

In all cases late blight did not reappear in destructive form until 3 to 5 days after the beginning of these 6 periods. Long periods of high humidity were necessary for the rapid spread and development of late blight. This point is further illustrated by the fact that even though the relative humidity was 100 per cent for 10 to 15 hours practically every night during the

TABLE 2.—*Maximum and minimum temperatures and rainfall preceding late blight epiphytotics on tomatoes in the winter of 1946*

| Period | Temperature, degrees F. | | Rainfall, inches |
|---|---|---|---|
| | Maximum | Minimum | |
| Jan. 14 | 80.0 | 53.9 | ..... |
| Jan. 15 | 80.0 | 55.6 | ..... |
| Jan. 16 | 69.3 | 66.3 | 0.53 |
| Jan. 21 | 79.8 | 62.0 | ..... |
| Jan. 22 | 80.4 | 58.8 | ..... |
| Jan. 23 | 56.2 | 51.2 | 0.73 |
| Feb. 27 | 73.4 | 63.2 | 0.03 |
| Feb. 28 | 74.8 | 62.2 | 3.19 |
| Mar. 1 | 67.8 | 61.0 | ..... |
| Mar. 2 | 69.6 | 57.1 | ..... |
| Mar. 13 | 69.1 | 60.0 | ..... |
| Mar. 14 | 65.2 | 59.5 | 1.10 |
| Mar. 15 | 82.9 | 59.2 | 0.26 |
| Mar. 27 | 85.3 | 62.1 | ..... |
| Mar. 28 | 77.4 | 58.4 | 0.29 |
| Mar. 29 | 78.5 | 65.6 | 0.04 |
| Apr. 16 | 70.8 | 57.0 | ..... |
| Apr. 17 | 80.8 | 65.2 | ..... |
| Apr. 18 | 71.0 | 61.5 | 0.18 |

entire season, late blight was relatively inactive except during the 6 periods mentioned. The reason for this quiescence of late blight during most of the season is probably due to the fact that the relative humidity usually dropped to 30 to 60 per cent for several hours every day. Crosier (2) and Crosier and Reddick (3) both have demonstrated that conidia of *Phytophthora infestans* rapidly lose their viability in atmosphere with the relative humidity below 95 per cent.

Climate and other factors in relation to the spread and development of late blight have been discussed by Bonde and Schultz (1), Crosier (2), Crosier and Reddick (3), Stakman and Christensen (7), and Thomas (9), but only insofar as they are related to the disease on potatoes. In keeping with these observations on potatoes, Kern and Orton (6) and Taubenhaus and Ezekiel (8) report that periods of low temperatures and frequent rainfall are necessary for late blight to be active and destructive on tomatoes.

The observations during the present epiphytotic clearly demonstrate that *Phytophthora infestans* may become active and destructive on tomatoes with temperatures of 60° to 70° F., provided there are long periods of 100 per cent relative humidity. The weather from January 21 to 24 and from February 27 to March 2 was particularly favorable for late blight since there were 65 and 75 hours respectively of continuous high humidity with moderately low temperatures. It was following these 2 periods that most of the damage was done to the plants in the seed beds and the young plants in the field.

## METHODS OF DISSEMINATION

Observational evidence during these epiphytotics clearly demonstrated that the spores of *Phytophthora infestans* might be borne for miles by the wind, provided the weather conditions were favorable. Isolated seed-beds and fields of tomatoes 30 and 40 miles from other tomatoes or potatoes were found to have late blight following some of these periods. The only possible way the fungus could have been carried to some of these fields was by the wind. The exact distances that the spores were carried or could be carried were not determined. They must have been carried 30 to 40 miles in some instances, especially to some of the fields in Collier County in south Florida. Some of the tomato fields in this county were planted miles from any habitation or other cultivated fields, yet late blight was found on tomatoes in some of these isolated fields in late January. The distance that the spores might be carried probably would depend on wind velocity, humidity, and temperature.

## SUMMARY

An unusual outbreak of late blight, *Phytophthora infestans*, is reported on tomatoes from the west coast of Florida. It started on the fall crop and became destructive in the winter seed-beds and continued throughout the spring season. There were 6 definite periods when the late-blight fungus

was particularly active. Rainfall was not so important in the spread and development of late blight as were long periods of high humidity. Late blight was relatively inactive unless the relative humidity was 100 per cent for more than 15 hours. Observational evidence indicates that spores of *Phytophthora infestans* were carried by winds 30 or more miles during certain periods when the weather was favorable.

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# BREEDING POTATOES FOR RESISTANCE TO RING ROT

REINER BONDE,[1] F. J. STEVENSON,[2] AND ROBERT V. AKELEY[3]

(Accepted for publication April 30, 1947)

## INTRODUCTION

The ring-rot disease caused by *Corynebacterium sepedonicum* (Spieck. and Kott.) Skap. and Burk. continues to be of major importance to the potato industry of the United States and Canada (7). Baribeau in 1931 (1) reported the disease as being prevalent in potato fields in the Province of Quebec and it was discovered the following year in the United States in a field of potatoes located in Aroostook County, Maine (2). Ring rot has spread rapidly and now is present in most of the potato growing areas of the United States and in some instances has caused very large losses (8).

Progress has been made in reducing the prevalence of bacterial ring rot in Maine and elsewhere. Most of the severely infested seed stocks in Maine have been discarded.[4] However, it still is prevalent and many seed stocks still have ring rot in trace amounts. It has been very difficult to eliminate traces of the disease from slightly infected seed stocks and often they have been responsible for very rapid spread of the disease. Furthermore, seed stocks with even a trace of ring rot are not acceptable as certified seed potatoes and a trace often causes an otherwise certifiable crop to be sold at greatly reduced prices.[5] Slightly infected seed stocks are a menace wherever susceptible varieties are grown.

The best solution to the ring-rot problem is to develop highly resistant or immune varieties. Work on the development of resistant varieties has been conducted for a number of years and the progress made is summarized in this paper.

## PRELIMINARY STUDIES

A search was started in 1939 to find varieties that possess resistance to ring-rot infection and that can serve as possible parents for use in a breeding program. The results of this preliminary investigation have been summarized by Bonde *et al.* (6).

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[4] Fields with 20 to 50 per cent infected plants were not uncommon in Maine in 1937 and 1938. Some growers discarded from 10 to 30 barrels of infected potatoes per acre and in some cases entire bins were badly decayed because of the prevalence of bacterial ring rot.

[5] The premium received for certified seed stocks over other seed stocks or table potatoes may be one dollar or more per hundred-weight. It is thus seen that the financial loss sustained by a farmer when a seed stock is rejected because of having a trace of ring rot may be very large.

For this work, 54 named domestic and foreign varieties and a number of unnamed seedling varieties from a wide range of sources were tested for resistance to infection by the ring-rot organism when heavily inoculated by artificial means. All but two of the named varieties were susceptible. The two resistant varieties were Friso and President (Paul Kruger), both from Holland. Two unnamed seedling selections included in this test also were highly resistant. One was from the progeny of a cross 41956 × Earlaine and the other from a cross Earlaine × 43055. The latter (46952) in addition to being resistant to ring rot is early in maturity, has attractive tubers, and is resistant to virus A in the field. Furthermore, it is self-fertile and will cross readily with other desirable varieties and selections.

Approximately 290 commercially desirable and selected seedling varieties of miscellaneous origin were tested for resistance to ring rot in other preliminary experiments. Seedling O55 (from progeny of Chippewa × Katahdin), 336–144 (from progeny of President × Katahdin), and 47102 (45146 × Earlaine) were the only ones in this experiment that failed to contract the disease.

## MATERIALS AND METHODS

In view of the above findings it seemed possible to produce desirable marketing varieties of potatoes that are resistant to ring rot and to other diseases.

The highly resistant varieties referred to previously were used in making crosses, and the progenies were tested for resistance to ring rot. In some cases only one resistant parent was used and in others both parents were resistant. Resistant varieties were crossed with varieties that are resistant to late blight and to the virus diseases for the purpose of combining these desirable qualities in a single variety or potential parent. The progenies of certain selfed lines also were tested, in the hope of developing new and better parents with increased resistance. The progenies from a number of miscellaneous crosses were included so that new sources of resistance and desirable parents might be found.

The use of pure cultures of the ring-rot organism for inoculations has not given reliable results in Maine. Inoculations with hypodermic needles and by some other methods likewise have been unsatisfactory. Therefore a rather simple method was employed for the inoculations included in these studies. The diseased portions of tubers infected with ring rot were thoroughly macerated and varying amounts of sterilized rain water were added to the infective material until a thick slurry resulted.[6] The tubers of the varieties under test were cut into seed pieces of appropriate size and dipped immediately into the water suspension of the bacteria. The inoculated seed pieces were planted immediately in the field.

[6] Infected stocks of the Katahdin variety were propagated for use as inoculum for these studies. Infected tubers of this variety disintegrate to a less extent than those of some of the other commercial varieties and have served as a good source of infective material for this kind of study.

The plants that emerged from the inoculated seed pieces were examined for foliage symptoms of ring rot during the growing season and were discarded if foliage symptoms appeared. The tubers from plants that developed no foliage symptoms of ring rot were dug after frost had killed the vines and the individual tubers were clipped at the stem end and carefully observed for signs of the disease. Those tubers not showing symptoms at harvest were put in storage and examined again prior to being planted the following spring.

## RESULTS

### *General Reaction to Ring Rot, and Other Characters, of Parents Used for Hybridizing*

The studies had indicated that a few potato varieties and seedlings are very resistant to ring rot. A study to test these for possible immunity was made during the four-year period from 1942 to 1945. The results have been summarized in table 1.

TABLE 1.—*Comparison of certain commercial and seedling potato varieties with respect to susceptibility to ring rot*[a]

| Variety | Parentage | Percentage of plants infected[b] | | | | Severity of decay in tubers |
|---|---|---|---|---|---|---|
| | | 1942 | 1943 | 1944 | 1945 | |
| Green Mountain | Excelsior × Dunmore | 82 | 90 | 95 | 80 | Severe; tubers disintegrated |
| Houma | Chas. Downing × Katahdin | 100 | 84 | 95 | 89 | Do |
| Katahdin | 40568 × 24642 | 80 | 65 | 72 | 68 | Severe; many tubers intact and firm |
| Sebago | Chippewa × Katahdin | ..... | 35 | 51 | 42 | Do |
| President[c] | Richter's Imperator × Wilhelm Korn | 0 | 0 | 2[d] | 1[d] | Very slight; confined to vascular ring |
| 47102[c] (Teton) | 45146 × Earlaine | 0 | 0 | 18[d] | 3[d] | Do |
| 46952[c] | Earlaine × 43055 | 2[d] | 3[d] | 2[d] | 3[d] | Slight |
| O55 | Chippewa × Katahdin | 5[d] | 4[d] | ..... | 4[d] | Do |
| 870[c] | Earlaine × 336–144 | 0 | 0 | 1[d] | 0 | Only slight softening in vascular ring in some tubers |
| 824[c] | Houma × 336–144 | 0 | 0 | 4[d] | 0 | Do |

[a] The freshly cut surfaces of seed pieces were inoculated by being dipped in a heavy water suspension of the bacteria and planted immediately in the field.

[b] Percentages based on 10 replicated lots of 10 seed pieces each for the different varieties.

[c] Same seed stock used for this study for four successive years.

[d] Ring rot suspected, but symptoms indeterminate. Slight infection in tubers after storage.

The Green Mountain, Houma, and Katahdin varieties were very susceptible to infection and developed high percentages of the disease for all of the four years. The Katahdin variety has appeared to be somewhat less susceptible to the disease than is the Green Mountain. However, in the field, the Katahdin variety often has the disease in rather large amounts.

This might be due to the fact that it is relatively resistant to the virus diseases and, therefore, is grown for longer periods of time by farmers before the seed stock is changed, thus allowing more time for the ring-rot disease to increase.

The Sebago variety, although here classified as susceptible, appears to possess some resistance to ring rot. This seems to be confirmed by results obtained by farmers, since surveys in Maine have shown that the Sebago

TABLE 2.—*Reaction to ring rot and desirable characteristics of named and seedling potato varieties used as parents*

| Variety | Reaction to ring rot[a] | Superior characters | |
|---|---|---|---|
| | | Disease resistance | Other characters |
| Chippewa | S | Mild mosaic virus A | Tuber shape, yield |
| Earlaine | S | ..... | Pollen fertility, earliness, tuber shape |
| Earlaine No. 2 | S | ..... | Yield |
| Friso | R | ..... | ..... |
| Green Mountain | S | ..... | Yield, cooking quality |
| Houma | S | ..... | Tuber shape, cooking quality |
| Katahdin | S | Mild mosaic virus A | Pollen fertility, tuber shape |
| Ostragis | S | Scab | ..... |
| President | R | Late blight | ..... |
| Sebago | S | Late blight, scab | ..... |
| Sequoia | S | Insects | Cooking quality |
| O55 | R | Southern brown rot | ..... |
| 76–7 | S | Late blight | ..... |
| 76–49 | S | Late blight | ..... |
| 96–28 | S | Late blight | ..... |
| 96–44 | S | Late blight | ..... |
| 96–56 | S | Late blight | ..... |
| 96–345 | S | Late blight | ..... |
| 336–18 | R | Late blight | ..... |
| 336–123 | S | Late blight | ..... |
| 336–144 | R | Late blight | ..... |
| 447–4 | S | ..... | ..... |
| 918–9 | S | Late blight | ..... |
| 918–12 | S | Late blight | ..... |
| 3895–3 | S | Late blight | Earliness |
| 3895–13 | S | Late blight | Earliness |
| 46952 | R | ..... | Pollen fertility, earliness, tuber shape |
| 47102 (Teton) | R | Virus X | Pollen fertility, tuber shape |

[a] Reactions were susceptible (S), or resistant (R) in tests for three or more successive years with inoculated seed stocks planted in the field.

seed stocks are relatively free of ring rot. Also, the symptoms of the disease are slow to manifest themselves in inoculated plants, and this too would indicate that this variety is somewhat resistant.

The President variety, as well as seedlings 47102 (recently named Teton), 46952, O55, 870, and 824 are resistant to ring rot and showed no infection, or very little, in the field (Table 1). These varieties, however, are not immune. Careful examination of the tubers from inoculated plants showed that the above-mentioned resistant varieties may become infected to a small degree with the symptoms in plants and tubers not very obvious. Seedlings

47102 and 46952 are self-fertile and now are used extensively in the breeding program for ring-rot resistance. President and O55 also have been used as parents. Seedlings 870 and 824 are probably the most resistant selections that have been made in Maine. The latter produced viable pollen in the greenhouse at Beltsville, Maryland, in 1946. Selfed seed was obtained and crosses made in which 824 was used as a pollen parent.

Twenty-eight parents were used in producing the progenies that were studied with regard to their resistance to ring rot. Table 2 summarizes the reactions of these parent varieties to ring-rot infection and gives some of their desirable characters. It may be noted from the data in table 2 that the 28 parents possess a wide range of characteristics that would be desirable to combine in varieties to be grown in Maine. It is of special value that Katahdin, 46952, and 47102 produce fertile pollen and readily fertilize most of the other varieties used as parents. Friso does not readily produce seed and requires special treatment and care when used for breeding purposes.

### *General Reaction of Progenies to Ring Rot*

Table 3 gives a summary of the reaction to ring rot of 49 different progenies, the parents of which have been listed and briefly described in table 2. An analysis of the data in table 3 shows that in the 13 progenies with both parents resistant, the percentage of resistant seedlings ranged from 53.0 to 89.9 and averaged 68.9; in the 28 progenies with one parent resistant, the range was 11.1 to 76.9 per cent and the average was 42.7 per cent; and in the 8 progenies with neither parent resistant, the range was 0 to 50.0 per cent and the average was 8.8 per cent.

It may be noted in table 2 that a number of the parents are susceptible to ring rot. The data in table 3 show that usually no resistant seedlings resulted when these parents were self-fertilized or were cross-fertilized with other susceptible varieties. The progenies listed in table 3 that produced no resistant seedlings are those from Chippewa selfed, Sebago × Ostragis, Sequoia × 96–56, 3895–3 × 447–4, 3895–13 × Earlaine, and 336–123 × 47156. Sebago appears to be somewhat resistant to ring rot, and a few resistant seedlings (20 per cent) resulted when it was hybridized with the susceptible variety, Earlaine. The progeny of 96–56 × Katahdin gave more resistant seedlings (50 per cent). Thus there is evidence that some susceptible varieties possess factors for ring-rot resistance that are transmitted to the progeny.

The seven ring-rot resistant varieties given in table 2 were hybridized with the different susceptible varieties that are listed. Twenty-eight progenies were obtained in which a susceptible and a resistant parent were used in making the crosses.

Resistance to ring rot was inherited and was transmitted to the progenies when one of the parents was resistant. The percentage of resistant seedling varieties that resulted from crossing a resistant parent with a susceptible one varied from 11.1 to 76.9 per cent, with an average of 42.7 per cent.

TABLE 3.—*General reactions of different progenies and Katahdin controls to ring rot, 1942 to 1945*

| Variety or cross and reaction to ring rot[a] | Seedlings or lots tested[b] | |
|---|---|---|
| | Number | Percentage resistant[c] |
| Katahdin (control) | 520 | 0.0 |
| Chippewa (S), Selfed | 110 | 0.0 |
| Chippewa (S) × 336–144 (R) | 120 | 40.2 |
| Chippewa (S) × 47102 (R) | 79 | 44.7 |
| Earlaine (S) × 336–144 (R) | 90 | 32.9 |
| Earlaine (S) × 46952 (R) | 132 | 45.5 |
| Earlaine No. 2 (S) × 46952 (R) | 9 | 11.1 |
| Friso (R) × Katahdin (S) | 120 | 45.8 |
| Green Mountain (S) × 336–144 (R) | 84 | 46.4 |
| Green Mountain (S) × 46952 (R) | 17 | 30.6 |
| Houma (S) × 336–144 (R) | 128 | 46.6 |
| President (R), Selfed | 127 | 54.4 |
| President (R) × Chippewa (S) | 18 | 40.0 |
| President (R) × Earlaine (S) | 353 | 38.2 |
| President (R) × Katahdin (S) | 336 | 43.2 |
| President (R) × 76–7 (S) | 15 | 40.0 |
| President (R) × 76–49 (S) | 95 | 43.2 |
| President (R) × 96–28 (S) | 214 | 43.6 |
| President (R) × 96–56 (S) | 89 | 44.9 |
| President (R) × 336–144 (R) | 140 | 74.7 |
| President (R) × 918–12 (S) | 80 | 66.0 |
| President (R) × 46952 (R) | 113 | 53.0 |
| President (R) × 47102 (R) | 17 | 64.7 |
| President (R) × 47156 (S) | 385 | 37.8 |
| Sebago (S) × Ostragis (S) | 18 | 0.0 |
| Sebago (S) × Earlaine (S) | 97 | 20.0 |
| Sebago (S) × 336–144 (R) | 15 | 47.0 |
| Sebago (S) × 46952 (R) | 112 | 13.0 |
| Sequoia (S) × 96–56 (S) | 12 | 0.0 |
| O55 (R) × 47102 (R) | 28 | 71.4 |
| 96–28 (S) × 336–144 (R) | 138 | 41.2 |
| 96–44 (S) × 336–144 (R) | 165 | 54.5 |
| 96–56 (S) × 336–144 (R) | 162 | 40.7 |
| 96–56 (S) × Katahdin (S) | 4 | 50.0 |
| 96–345 (S) × 336–144 (R) | 187 | 47.8 |
| 336–18 (R), Selfed | 103 | 81.4 |
| 336–18 (R) × 96–56 (S) | 217 | 52.5 |
| 336–18 (R) × 46952 (R) | 12 | 58.3 |
| 336–123 (S) × 47156 (S) | 20 | 0.0 |
| 336–144 (R), Selfed | 60 | 71.0 |
| 918–9 (S) × 46952 (R) | 68 | 44.0 |
| 3895–3 (S) × 447–4 (S) | 94 | 0.0 |
| 3895–13 (S) × Earlaine (S) | 22 | 0.0 |
| 46952 (R), Selfed | 39 | 66.2 |
| 46952 (R) × 336–144 (R) | 184 | 53.7 |
| 46952 (R) × 47102 (R) | 79 | 89.9 |
| 46952 (R) × Katahdin (S) | 5 | 40.0 |
| 47102 (Teton) (R), Selfed | 149 | 85.0 |
| 47102 (R) × 96–56 (S) | 195 | 76.9 |
| 47102 (R) × 336–144 (R) | 25 | 72.0 |

[a] (S) indicates that parent is susceptible and (R) that parent is resistant.

[b] From 5 to 10 freshly cut seed pieces of each seedling or of each Katahdin lot were inoculated by being dipped in a heavy suspension of the bacteria and were then planted immediately in the field.

[c] Those classed as resistant showed no symptoms of ring rot in foliage or tubers.

The variety President was one of the first that was found in Maine to be resistant to ring rot and it has been used extensively for producing some of the progenies used in these studies. Approximately 1725 seedlings secured by crossing President and a susceptible variety were tested for resistance to ring rot. Of these 773, or 44.8 per cent, were resistant.

Seedlings 336–18 and 336–144 which were secured from the progeny of President × Katahdin are resistant to ring rot and late blight and have produced good yields in Maine. Two hundred seventeen seedlings, having as parents 336–18 and the ring-rot-susceptible seedling variety 96–56, were included in the test, of which 114, or 52.5 per cent, were resistant. The resistance in 336–144 also was transmitted. There were 1089 seedlings tested that had 336–144 and some susceptible variety as parents, of which 489, or 44.9 per cent, failed to contract ring rot. It is of interest here that 336–123, which also has President and Katahdin as parents, is resistant to late blight and is phenotypically similar to 336–18 and 336–144 but is very susceptible to ring rot. No ring-rot-resistant seedlings were obtained in the progeny when 336–123 was hybridized with susceptible 47156.

Friso and 47102 also produce progenies with a high percentage of resistant seedlings when they are used for making crosses (Table 3). Friso crossed with the susceptible Katahdin gave 120 seedlings of which 55, or 45.8 per cent, were resistant to ring rot. When 47102 was crossed with the susceptible Chippewa variety, 44.7 per cent were resistant and when it was crossed with 96–56, 76.9 per cent were resistant.

Seedling 46952, although a desirable parent for breeding purposes, has not been considered as resistant to ring rot as some of the other selections that were used. Of 343 seedlings tested that had 46952 and some susceptible variety as parents, 105, or 30.6 per cent, possessed resistance to the disease.

The data also show that resistance to ring rot is accumulative and can be increased by combining the factors from different parents. When the resistant parents President, 336–18, 336–144, 46952, and 47102 were self-fertilized, from 54.4 to 85.0 per cent of the seedlings in the progenies were resistant. Likewise, when both of the parents were resistant, from 53 to 89.9 per cent of the seedlings were resistant.

### *The Percentage of Inoculated Seed Pieces Producing Plants with Symptoms*

The ring-rot infection that occurred in the parents and progenies of 33 crosses tested in 1944 and 1945 is given in table 4. The extent of ring-rot infection is shown in table 4 in five classes based on the percentage of plants in each lot that contracted the disease.

The lots in Class 0 developed no infection as a result of the inoculations and are considered as being highly resistant. Those in Class 1 had some degree of resistance inasmuch as only one or two plants out of ten became infected and the degree of infection in the tubers also was slight. The lots

TABLE 4.—*Percentage and severity of infection in plants from inoculated seed pieces of Katahdin controls, parents of crosses, and their progenies, in 1944 and 1945*[a]

| Variety or cross and reaction to ring rot[b] | Lots tested | Percentage of lots in each infection class[c, d] | | | | | | Lots infected |
|---|---|---|---|---|---|---|---|---|
| | | Class 0 | Class 1 | Class 2 | Class 3 | Class 4 | Class 5 | |
| *Controls and parent lots* | *No.* | *Pct.* | *Pct.* | *Pct.* | *Pct.* | *Pct.* | *Pct.* | *Per cent* |
| Katahdin controls | 500 | ..... | ..... | 4 | 6 | 50 | 40 | 100 |
| Chippewa (S) | 20 | ..... | ..... | ..... | ..... | 20 | 80 | 100 |
| Earlaine (S) | 20 | ..... | ..... | ..... | ..... | ..... | 100 | 100 |
| Earlaine No. 2 (S) | 20 | ..... | ..... | ..... | ..... | ..... | 100 | 100 |
| Green Mountain (S) | 20 | ..... | ..... | ..... | ..... | 20 | 80 | 100 |
| Houma (S) | 20 | ..... | ..... | ..... | ..... | ..... | 100 | 100 |
| President (R) | 20 | 100 | ..... | ..... | ..... | ..... | ..... | 0 |
| Sebago (S) | 20 | ..... | 10 | 20 | 20 | 30 | 20 | 100 |
| Sequoia (S) | 20 | ..... | ..... | 20 | 60 | 20 | ..... | 100 |
| O55 (R) | 10 | 90 | 10 | ..... | ..... | ..... | ..... | 10 |
| B76–7 (S) | 10 | ..... | 10 | 20 | 40 | 10 | 20 | 100 |
| B76–49 (S) | 20 | ..... | 10 | 20 | 50 | 20 | 0 | 100 |
| 96–28 (S) | 20 | ..... | ..... | ..... | ..... | 10 | 90 | 100 |
| 96–44 (S) | 20 | ..... | ..... | 20 | ..... | 10 | 70 | 100 |
| 96–56 (S) | 20 | ..... | ..... | ..... | ..... | ..... | 100 | 100 |
| 96–345 (S) | 20 | ..... | ..... | ..... | ..... | 20 | 80 | 100 |
| 336–18 (R) | 10 | 100 | ..... | ..... | ..... | ..... | ..... | 0 |
| 336–144 (R) | 20 | 100 | ..... | ..... | ..... | ..... | ..... | 0 |
| 46952 (R) | 20 | 90 | 10 | ..... | ..... | ..... | ..... | 10 |
| 47102 (Teton) (R) | 50 | 100 | ..... | ..... | ..... | ..... | ..... | 0 |
| 47156 (S) | 10 | ..... | ..... | ..... | ..... | ..... | 100 | 100 |
| *Progeny lots* | | | | | | | | |
| Chippewa (S) × 47102 (R) | 79 | 75 | 11 | 5 | 4 | 3 | 3 | 26 |
| Earlaine (S) × 336–144 (R) | 56 | 47 | 14 | 11 | 7 | 2 | 20 | 54 |
| Earlaine (S) × 46952 (R) | 49 | 53 | 0 | 12 | 10 | 4 | 20 | 46 |
| Earlaine No. 2 (S) × 46952 (R) | 9 | 11 | 0 | 11 | 22 | 11 | 45 | 89 |
| Green Mountain (S) × 336–144 (R) | 84 | 45 | 5 | 5 | 4 | 1 | 41 | 56 |
| Green Mountain (S) × 46952 (R) | 17 | 71 | 12 | 0 | 12 | 0 | 6 | 30 |
| Houma (S) × 336–144 (R) | 69 | 54 | 3 | 1 | 3 | 3 | 36 | 46 |
| Sebago (S) × 46952 (R) | 57 | 5 | 4 | 5 | 7 | 4 | 76 | 96 |
| Sequoia (S) × 96–56 (S) | 6 | 0 | 17 | 0 | 17 | 0 | 66 | 100 |
| President (R) × Self | 113 | 55 | 13 | 11 | 5 | 6 | 10 | 45 |
| President (R) × Katahdin (S) | 106 | 31 | 7 | 17 | 7 | 2 | 37 | 70 |
| President (R) × B76–7 (S) | 15 | 40 | 27 | 13 | 7 | 0 | 13 | 60 |
| President (R) × B76–49 (S) | 95 | 43 | 21 | 7 | 7 | 2 | 19 | 56 |
| President (R) × 96–28 (S) | 215 | 43 | 7 | 10 | 7 | 6 | 28 | 58 |
| President (R) × 96–46 (S) | 89 | 45 | 17 | 7 | 8 | 9 | 15 | 56 |
| President (R) × 47102 (Teton) (R) | 17 | 65 | 18 | 6 | 6 | 0 | 6 | 36 |
| President (R) × 47156 (S) | 108 | 55 | 4 | 7 | 1 | 2 | 31 | 45 |
| O55 (R) × 47102 (R) | 28 | 71 | 14 | 4 | 4 | 4 | 4 | 30 |
| 96–28 (S) × 336–144 (R) | 138 | 41 | 12 | 7 | 8 | 6 | 26 | 59 |
| 96–44 (S) × 336–144 (R) | 165 | 55 | 7 | 3 | 7 | 3 | 26 | 46 |
| 96–56 (S) × 336–144 (R) | 64 | 31 | 2 | 5 | 8 | 13 | 42 | 70 |
| 96–345 (S) × 336–144 (R) | 107 | 56 | 12 | 1 | 3 | 5 | 23 | 44 |
| 336–18 (R) × Self | 77 | 82 | 16 | 1 | 1 | 0 | 0 | 18 |
| 336–18 (R) × 96–56 (S) | 205 | 47 | 17 | 10 | 12 | 4 | 10 | 53 |
| 336–18 (R) × 46952 (R) | 12 | 58 | 17 | 17 | 8 | 0 | 0 | 42 |
| 336–144 (R) × Self | 24 | 79 | 0 | 0 | 4 | 8 | 8 | 20 |
| 46952 (R) × Self | 22 | 77 | 9 | 9 | 0 | 0 | 5 | 23 |
| 46952 (R) × 336–144 (R) | 42 | 54 | 2 | 3 | 4 | 4 | 32 | 45 |
| 46952 (R) × 47102 (R) | 71 | 92 | 6 | 0 | 0 | 0 | 2 | 8 |
| 46952 (R) × Katahdin (S) | 5 | 20 | 40 | 0 | 0 | 0 | 40 | 80 |

TABLE 4.—(*Continued*)

| Variety or cross and reaction to ring rot[b] | Lots tested | Percentage of lots in each infection class[c, d] | | | | | | Lots infected |
|---|---|---|---|---|---|---|---|---|
| | | Class 0 | Class 1 | Class 2 | Class 3 | Class 4 | Class 5 | |
| *Progeny lots* (Continued) | *No.* | *Pct.* | *Pct.* | *Pct.* | *Pct.* | *Pct.* | *Pct.* | *Per cent* |
| 47102 (R) × Self | 149 | 85 | 8 | 2 | 1 | 1 | 4 | 16 |
| 47102 (R) × 96–56 (S) | 195 | 77 | 8 | 6 | 4 | 3 | 2 | 23 |
| 47102 (R) × 336–144 (R) | 25 | 72 | 24 | 0 | 0 | 0 | 4 | 28 |

[a] Ten freshly cut seed pieces of each variety or lot were inoculated by being dipped in a heavy suspension of the bacteria and were planted immediately in the field.

[b] (S) indicates that parent is susceptible and (R) that parent is resistant.

[c] Class of infection — Plants infected

| | |
|---|---|
| 0 | None |
| 1 | 1–2 slightly infected |
| 2 | 3–4 severely infected |
| 3 | 5–6 severely infected |
| 4 | 7–8 severely infected |
| 5 | 9–10 severely infected. |

[d] Total in all classes will not always be 100 per cent, due to use of round numbers.

listed in Classes 2, 3, 4, and 5 are considered definitely susceptible, 3 to 10 plants out of 10 becoming infected and the severity of infection as measured by the amount of rot in the tubers also being great.

Katahdin controls and the parents Chippewa, Earlaine, Earlaine No. 2, Green Mountain, Houma, Sequoia, 96–28, 96–44, 96–56, 96–345, and 47156 are all definitely susceptible to ring rot, with no lots in Classes 0 and 1 (Table 4). Sebago, B76–7, and B76–49 possessed some resistance as is shown by the fact that some lots were in Class 1. In President, 336–18, 336–144, and 47102 all of the lots were in Class 0, which indicates considerable resistance. Seedlings O55 and 46952 were somewhat less resistant, some lots being in Class 1.

The data in table 4 confirm those presented in table 3 and show that a high percentage of resistant seedlings occur in the progenies when one or both of the parents are resistant to the disease. From 5 to 77 per cent (average 45) of the lots in the different progenies were in the resistant Class 0 when one parent was resistant, and from 54 to 92 per cent (average 71.8) were in Class 0 when both parents were resistant, including cases where the resistant parents were self-fertilized. When President, 336–18, 336–144, 46952, and 47102 were selfed, 55, 82, 79, 77, and 85 per cent, respectively, of the seedlings in the progenies were resistant and in Class 0. Furthermore, an additional number was in Class 1 and showed only a slight degree of infection.

### *Reliability of Ring-Rot Inoculation of Seedlings Selected as Being Resistant*

The question naturally arises whether the seedlings classified as resistant in tables 3 and 4 are actually resistant to ring rot or whether they merely escaped infection when artificially inoculated.

Some data were secured that pertain to this problem. Three hundred forty-seven seedlings that were selected as resistant in 1944 were reinoculated in 1945 and observations made on the development of the disease.

It was observed that 286 or 82.42 per cent of the 347 seedlings found to be free of ring rot in 1944 continued to be free of infection after inoculations in 1945. There were very few actual "escapes." Most of the seedlings that became infected in 1945 contracted the disease in relatively few plants or tubers. Furthermore, in many cases the degree of infection was slight and the bacteria were localized in relatively small areas of the tubers. The disease also was difficult to detect in some seedlings, because only very slight symptoms were apparent.

The writers believe that the method used for testing resistance to ring rot is reliable. Most of the highly susceptible seedlings are readily detected and those that survive the test have varying degrees of resistance.

### *Severity of Infection in Tubers in Progenies from Crosses Including Resistant Varieties*

Studies described above have shown that the progenies resulting from crossing certain ring-rot-resistant and susceptible varieties contain high percentages of new seedling varieties that are resistant to the disease. The results were based on the presence or absence of foliage and tuber symptoms of the disease, no attention being paid to the extent and severity of the tuber rot that resulted.

An experiment was conducted to ascertain if resistance to ring rot in the tubers, based on percentage of tubers infected and severity of decay, also is transmitted to the progenies when resistant parents are used for making crosses.

Tubers from inoculated plants of 410 resistant seedlings selected from 12 crosses were put into storage and carefully examined for the presence of ring rot the following spring. The seedlings were graded into five classes according to the percentage of tubers infected and the severity of decay. Class 0 had no infection. In Class 1 there was a trace of infection in less than 25 per cent of the tubers. In Class 2 there was a trace of infection in 26 to 50 per cent of the tubers. In Class 3, 51 to 75 per cent of the tubers were infected and the decay was usually medium in severity. In Class 4, 76 to 100 per cent of the tubers were infected and the decay usually was very severe.

The data are summarized in table 5 and show that the tubers of the parent varieties Katahdin, Chippewa, Earlaine, Houma, 96–56, and 96–345 were included mostly in the highly susceptible Classes 3 and 4. The Sebago variety was somewhat less susceptible to ring rot and 15 per cent of the tubers showed no symptoms of the disease.

On the other hand, the parent varieties 46952, Friso, President, and 336–144 were resistant to ring rot, with from 70 to 75 per cent of the tubers disease-free and included in Class 0. From 10 to 20 per cent of the remaining tubers developed only a slight degree of rot.

Resistance to ring rot in the tubers was transmitted to the progeny when the resistant parents were used for making the crosses.

From 37 to 75 per cent of the progenies showed no tuber decay and were included in Class 0 when seedling 336–144 was used as one parent in making crosses with susceptible varieties. In addition, from 7 to 35 per cent of these progenies developed a very slight degree of infection, being in Class 1. The progenies with no tuber infection were increased to 83 per cent when the resistant parent 336–144 was crossed with the somewhat resistant parent 46952.

Tuber resistance in the varieties President, Friso, and 46952 likewise was transmitted to the progenies when these three varieties were used as parents in making the crosses (Table 5).

The reaction to tuber decay of the seedlings selected as being resistant

TABLE 5.—*Resistance to tuber infection of controls, parents, and resistant selections from different progenies. Crop of 1944*

| Variety or cross and reaction to ring rot[a] | Lots tested[b] | Lots in each class of infection[c] | | | | | Lots infected |
|---|---|---|---|---|---|---|---|
| | | Class 0 | Class 1 | Class 2 | Class 3 | Class 4 | |
| | *No.* | *Pct.* | *Pct.* | *Pct.* | *Pct.* | *Pct.* | *Per cent* |
| *Controls and parent lots* | | | | | | | |
| Green Mountain controls | 200[d] | 0 | 0 | 0 | 40 | 60 | 100 |
| Katahdin (S) | 20 | 0 | 0 | 10 | 30 | 40 | 80 |
| Chippewa (S) | 20 | 0 | 0 | 5 | 25 | 50 | 80 |
| Sebago (S) | 20 | 15 | 5 | 10 | 20 | 10 | 45 |
| Earlaine (S) | 20 | 0 | 0 | 0 | 20 | 50 | 70 |
| Houma (S) | 20 | 0 | 0 | 10 | 35 | 40 | 85 |
| 96–56 (S) | 20 | 0 | 0 | 15 | 40 | 30 | 85 |
| 96–345 (S) | 20 | 0 | 0 | 25 | 30 | 25 | 80 |
| 46952 (R) | 20 | 70 | 20 | 0 | 0 | 0 | 20 |
| Friso (R) | 20 | 75 | 10 | 0 | 0 | 0 | 10 |
| President (R) | 20 | 70 | 20 | 0 | 0 | 0 | 20 |
| 336–144 (R) | 20 | 70 | 10 | 0 | 0 | 0 | 10 |
| *Progeny lots* | | | | | | | |
| Chippewa (S) × 336–144 (R) | 46 | 37 | 35 | 15 | 11 | 2 | 63 |
| President (R) × 336–144 (R) | 63 | 70 | 24 | 6 | 0 | 0 | 30 |
| Houma (S) × 336–144 (R) | 23 | 74 | 26 | 0 | 0 | 0 | 26 |
| 96–56 (S) × 336–144 (R) | 33 | 75 | 19 | 3 | 3 | 0 | 25 |
| 96–345 (S) × 336–144 (R) | 29 | 75 | 7 | 13 | 4 | 0 | 24 |
| 46952 (R) × 336–144 (R) | 12 | 83 | 17 | 0 | 0 | 0 | 17 |
| Sebago (S) × Earlaine (S) | 6 | 50 | 17 | 17 | 17 | 0 | 51 |
| President (R) × Earlaine (S) | 49 | 75 | 19 | 6 | 0 | 0 | 25 |
| President (R) × Katahdin (S) | 80 | 45 | 27 | 22 | 5 | 1 | 55 |
| Sebago (S) × 46952 (R) | 4 | 50 | 25 | 0 | 0 | 25 | 50 |
| Earlaine (S) × 46952 (R) | 15 | 67 | 27 | 7 | 0 | 0 | 34 |
| Friso (R) × Katahdin (S) | 50 | 52 | 28 | 14 | 6 | 0 | 48 |

[a] (S) indicates that parent is susceptible and (R) that parent is resistant.

[b] Five seed pieces for each lot or seedling tested were inoculated by being dipped in a heavy suspension of bacteria in water and were planted immediately in the field.

[c] Based on symptoms in tubers cut after being in storage: 0 = no infection on tubers when cut; 1 = trace infection in 1 to 25 per cent of tubers; 2 = trace infection in 26 to 50 per cent of tubers; 3 = infection in 51 to 75 per cent of tubers, with decay usually medium in severity; 4 = infection in 76 to 100 per cent of tubers, with decay usually very severe.

[d] Used tubers from 200 infected plants.

to ring rot was in striking contrast to that which developed in the Green Mountain controls. The tubers of 200 Green Mountain plants infected with ring-rot bacteria likewise were placed in storage and examined. All tubers had the disease, with a high percentage completely disintegrated when examined. All of the tubers from the infected Katahdin plants also had the disease, but the extent of the decay was less severe and many of the tubers were still relatively firm and intact.

### *Combination of Ring-Rot Resistance with Desirable Tuber Type and Other Marketing Qualities*

The seedlings from 12 progenies were inoculated with ring-rot bacteria for two successive years, and the percentage of resistant seedlings for each year was recorded. The seedling varieties that became infected were discarded each season, as also were those that had undesirable tuber type or plant characteristics. Table 6 summarizes the results.

TABLE 6.—*Proportion of ring-rot-resistant seedlings possessing desirable marketing qualities and plant characteristics in 1944 and 1945*

| Parentage | Seedlings resistant to ring rot in field test[a] | Resistant seedlings with desirable marketing qualities | |
|---|---|---|---|
| | *No.* | *No.* | *Per cent* |
| Sebago × Earlaine | 98 | 1 | 1.0 |
| President × Earlaine | 180 | 1 | 0.5 |
| President × 336–144 | 175 | 3 | 0.6 |
| President × Katahdin | 125 | 4 | 3.2 |
| Houma × 336–144 | 60 | 2 | 3.3 |
| 336–144 selfed | 27 | 0 | 0.0 |
| 336–18 selfed | 77 | 0 | 0.0 |
| Friso × Katahdin | 120 | 26 | 26.7 |
| 96–345 × 336–144 | 120 | 20 | 16.7 |
| 46952 × 47102 | 71 | 50 | 70.0 |
| Chippewa × 47102 (Teton) | 79 | 32 | 40.0 |
| 47102 (Teton) × 96–56 | 195 | 63 | 32.3 |
| Katahdin controls | 125 | 0 | 0.0 |

[a] Freshly cut seed pieces of each lot were inoculated with ring-rot bacteria and were planted in the field. Only those are included here that were resistant to ring rot.

All of the Katahdin controls developed ring rot when inoculated with the pathogen (Table 6). Relatively few seedlings from the progenies of Sebago × Earlaine, President × Earlaine, President × 336–144, Houma × 336–144, and President × Katahdin possessed good tuber type and also remained free from ring rot for the two seasons. It was shown in previous experiments that the President variety and the related seedlings 336–18 and 336–144 when used as parents produce progenies with a high percentage of resistant seedlings. However, many of these seedlings, although resistant to ring rot, have excessively long stolons, and are too late in maturing to be considered suitable for Maine. Moreover, many of the tubers are of poor shape or cling too tightly to the vines. Therefore President and other re-

lated varieties, including 336–18 and 336–144, are not desirable parents for the production of new varieties that will be adapted to Maine.

Fortunately, however, there are other parents that transmit ring-rot resistance, along with desirable tuber type and other marketing qualities. It may be noted in table 6 that when Friso was crossed with Katahdin, nearly 27 per cent of the progeny were resistant to ring rot and also possessed desirable plant characteristics and tuber type. Friso, however, is not self-fertile and does not readily retain its flowers when fertilized by other varieties and, therefore, is not a desirable parent. When 96–345 was crossed with 336–144, approximately 17 per cent of the seedlings were resistant and otherwise desirable. It appears that 96–345, which is resistant to late blight, is a good parent to use in combination with other seedlings that are resistant to ring rot.

Seedlings 46952 and 47102 are resistant parents with viable pollen. When these parents were used for making crosses, a high percentage of the seedlings in the progenies were not only resistant to ring rot but also possessed attractive tubers and plants with desirable cultural characteristics. When 46952 was crossed with 47102, both of which are resistant, 70 per cent of the progeny also were resistant. Furthermore, none of the seedlings in the progenies were excessively late in maturing and the tubers in many cases were attractive. When 47102 was crossed with the susceptible varieties Chippewa and 96-56, 40.0 and 32.3 per cent, respectively, of the seedlings in the progenies were resistant to ring rot and many of these had attractive tubers and were relatively early in maturity.

### *Comparison of Yields of Ring-Rot-Resistant Seedlings With Yields of Standard Varieties*

A relatively large number of ring-rot-resistant seedlings have been developed in Maine as a result of the breeding program. Most of these are of rather recent origin and have not been studied critically regarding their performance in the field.

Data were secured in 1945 on the yields of four ring-rot-resistant seedling varieties in comparison with Pawnee, a newly named variety, and with four standard varieties now being grown in Aroostook County, Maine (Table 7).

Seedling 47102 gave the highest yield among the nine varieties tested. It also was superior in the general appearance of the crop, the tubers being uniform in size and having an attractive white color. It, however, is somewhat less smooth than some of the newer varieties that recently have been developed. This seedling has been described by Starr and Riedl as being resistant to ring rot and as possessing good cooking and keeping qualities (9, p. 14). Studies conducted in Maine confirm the results of Starr and Riedl that this variety is very resistant (3, p. 508; 4, p. 214; and 5, p. 421). It has contracted only a trace of the disease, over six successive years, even after freshly cut seed pieces were heavily contaminated with viable ring-rot bacteria prior to being planted in the field. The successive seed stocks

grown from seed pieces of this variety inoculated with the disease organisms in 1941 and 1942 have shown no apparent symptoms even at this date. In contrast, all of the tubers and plants from inoculated seed stocks of the Katahdin and Green Mountain varieties developed symptoms of ring rot.

In one yield test Green Mountain, long considered a high yielding variety in Maine, produced 15 barrels or 41 bushels less per acre than did seedling 47102. Furthermore, 20 per cent of the Green Mountain tubers developed leafroll net necrosis, a condition that was not noted in the new seedling variety.

Seedling 336–144 has been resistant to ring rot, has produced good yields, and possesses some resistance to late blight. It, however, is too late

TABLE 7.—*Yields of four ring-rot-resistant seedling varieties compared with Pawnee and four standard varieties*

| Variety or selection | Characteristics of varieties | Yields per acre[a] | |
|---|---|---|---|
| | | *Barrels* | *Bushels* |
| O55 | Late, resistant to ring rot, with good tuber shape | 119 | 328 |
| 46952 | Early, resistant to ring rot, with good tuber shape | 114 | 314 |
| 336–144[b] | Very late, resistant to ring rot, somewhat resistant to late blight | 142 | 418 |
| 47102 (Teton) | Medium late, resistant to ring rot, yielding well | 178 | 492 |
| Pawnee | Medium early, promising for Greeley, Colorado, and other areas | 154 | 424 |
| Green Mountain | Late standard variety for Maine, with good cooking quality | 163 | 451 |
| Katahdin | Late standard variety for Maine | 116 | 320 |
| Sebago | Late standard variety for Maine | 137 | 377 |
| Irish Cobbler | Early standard variety for Maine | 137 | 377 |

[a] Based on six replicated plots of 25 hills each for each variety. Minimum required for significance is 29.1 barrels or 80.0 bushels at the 1 per cent level and 21.7 barrels or 59.7 bushels at the 5 per cent level.

[b] Seed stock had 20 per cent leaf roll.

in maturing to be grown commercially in northern Maine. It also appears to be very susceptible to leafroll.

Seedlings O55 and 46952, which are resistant to ring rot, gave rather low yields in these tests. However, 46952 possesses viable pollen and produces seedlings with attractive tubers when used as a parent in crosses with other varieties.

It may be noted that the Katahdin variety yielded only 116 barrels, or 320 bushels, per acre in these tests. This variety often has produced rather low yields in Maine in comparison with some of the older varieties.

It would seem to be possible to develop new varieties that would consistently yield more than the Katahdin, be resistant to ring rot and the other diseases, and also possess good cooking and marketing qualities.

## DISCUSSION

Resistance to ring rot has been found in relatively few varieties. These varieties were obtained originally from a number of widely separated sources, namely Friso and President from the Netherlands and a number of unrelated seedling varieties produced in the United States (6). More recently, resistance has been found in a number of other unrelated selections. Since ring-rot resistance has been found in such unrelated material it would seem desirable to test in the future more varieties from different sources to make additional selections that may be used for producing varieties that are resistant to ring rot.

A number of varieties and seedlings used as parents, as well as the progenies from different crosses, were tested for resistance to ring rot when inoculated by artificial methods. The data show that no, or very few, resistant seedlings are produced when certain susceptible parents are intercrossed. However, when parents known to carry factors for resistance are crossed with susceptible varieties, a large number of resistant seedlings appear in the progenies. The relative number of resistant seedlings that are produced is increased further if the resistant selections are inbred or are crossed with other resistant varieties.

Resistance to ring rot is inherited and it is possible to produce resistant varieties with desirable marketing qualities. President, seedlings 336–144 and 336–18 (both from the cross President × Katahdin), and other related parents transmit a high percentage of resistance to the progenies. However, most of these resistant seedlings are very late, have ill-shaped tubers, and are otherwise undesirable. Because of this, earlier resistant varieties are now being used as parents for making crosses. Seedlings 46952 and 47102 transmit ring-rot resistance and also produce seedlings that possess good cooking and marketing qualities.

Furthermore, when these resistant parents are crossed with selections from U.S.D.A. crosses 76, 96, and 918, which are resistant to late blight, a large percentage of the seedlings in the progenies also is highly resistant to late blight, besides being resistant to ring rot.

Degrees of resistance and susceptibility were apparent throughout the tests. Some of the selections were very resistant and no, or very few, diseased plants or tubers resulted because of the inoculations. Other seedlings became infected but in a relatively small percentage of the plants and tubers. Some were tolerant to ring rot and developed only slight symptoms of the disease, and the tubers remained relatively firm and intact even when infected. There also were some that manifested severe symptoms in that the plants wilted and died rapidly and the tubers soon disintegrated.

According to the data in this paper, the resistant seedling varieties that have been developed are not immune from the disease. The symptoms in some cases are not distinct and the plants are rather tolerant. It may be justly asked whether such varieties will be desirable and whether they may not serve as perpetual sources of infection.

The writers believe that highly resistant varieties will be of value even if they are not immune. The results presented are based on artificial inoculations which were much more severe than the contamination that may normally occur in the field or in the farmers' storage houses. The controls and other commercial varieties were all infected by the first year's inoculations. However, inoculation of certain resistant varieties resulted in none or small percentages of infection, and a repetition of inoculation in the three succeeding years also resulted in very few infections. One resistant variety has been tested for six successive years with only a trace of ring rot resulting. The writers have concluded that some of the resistant varieties discussed here would not have become infected with ring rot under normal farm conditions. One of these (47102) has recently been named "Teton" and has been distributed to a number of growers.

## SUMMARY

Ring rot, caused by *Corynebacterium sepedonicum* (Spieck. and Kott.) Skap. and Burk., continues to be a major potato disease. Many growers have been unable to secure and maintain healthy seed stocks and in many cases have suffered large losses. The availability of resistant or immune varieties would help greatly to control ring rot.

The resistant variety President and five resistant seedling selections were severely inoculated with ring-rot bacteria and tested for resistance to ring rot for four successive years. These varieties contracted very little disease as a result of the tests. In contrast, the susceptible varieties developed a high percentage of infection and none survived the test for more than one year.

Forty-nine progenies derived from crosses involving resistant and susceptible parents were tested for resistance to ring rot. No resistant seedlings, or very few, occurred in the progenies when both of the parents were susceptible. A relatively high percentage of a progeny was resistant when one or both parents were resistant. The percentage of resistant seedlings was increased when both of the parents were resistant or when resistant parents were self-fertilized.

The variety President and the related selections (336–18, 336–144, etc.) produced high percentages of ring-rot-resistant seedlings when used in the breeding program. These seedlings, however, are too late in maturing and their tubers are too ill-shaped for them to be of value as commercial varieties.

Seedlings 46952 and 47102 are resistant and have viable pollen. They produced progenies with high percentages of resistant seedlings that have good marketing quality.

Friso, a Dutch variety, also produced a high percentage of resistant seedlings with good marketing qualities but is pollen-sterile and does not readily produce viable seed.

The method used in this study for testing the seedlings for resistance to

ring rot was found to be fairly accurate. There were very few actual escapes and plants that survived the test in most cases possessed a high degree of resistance.

Resistance of the tubers to ring-rot decay also was transmitted to the progenies when resistant parents were used for making the crosses.

When the ring-rot-resistant seedlings 46952 and 47102 were crossed with certain parents that are resistant to late blight, a number of selections were secured that are highly resistant to both diseases.

Seedling 47102, which is very resistant to ring rot, gave the highest yield in 1945 in comparison with five standard varieties and three new seedling varieties. This selection has been named Teton and distributed to a number of growers.

With the desirable parent material now available, it should not be difficult to produce new varieties with good marketing qualities that also are resistant to ring rot and other diseases. It appears to be chiefly a matter of making the necessary crosses, selecting from the progenies the seedlings that possess the desirable characters, and testing them for resistance to ring rot and other diseases.

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# WHEAT DWARF BUNT DEPRESSED BY COMMON BUNT[1]

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## INTRODUCTION

Common bunt (*Tilletia foetida* (Wallr.) Liro and *T. caries* (DC.) Tul.) and dwarf bunt (*T. caries*)[3,4,5] frequently occur in the same wheat field but only occasionally do both kinds develop on the same wheat plant. That the two kinds do not occur together on the same plant more commonly suggests that their developmental processes are antagonistic to each other, either at the time of infection or during development within the host. The results of experiments to determine whether there is a depressing effect of one of these kinds of bunt upon the other are presented in this paper.

## MATERIALS AND METHODS

Four tests were made, one of which was in Utah and the others in Montana. Since dwarf bunt cannot be produced by seed inoculation these tests were made in commercial wheat fields in Utah and Montana where the soil was naturally contaminated with dwarf bunt spores. Some of the seed that was planted was inoculated with spores of common bunt while some was not inoculated. Inoculated and noninoculated seed was planted in adjacent rows in the dwarf bunt-infested soil. In the one test in Utah the percentage of smut was determined on the basis of plant counts and in the others in Montana by head counts.

The following wheat varieties were used: Hybrid 128 (C.I. 4512[6]), Oro (C.I. 8220), Golden (C.I. 10063), Yogo (C.I. 8033), and Utah Kanred (C.I. 11608). The inoculum included dwarf bunt (in the soil), and spores of races L–1, 2, 7, and 8 of *Tilletia foetida* and T–2, 10, and 16 of *T. caries*.[7] The powdered inoculum was applied to the seed at the rate of about one gm. spores to 100 gm. seed.

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## EXPERIMENTAL RESULTS

Utah Kanred wheat and race L–2 were used for the test in Utah. Plantings were made in dwarf bunt-infested soil near Clarkston, Utah, on October 1, 1941, and duplicated on an adjacent plot 16 days later. There were 24 eight-foot rows in each planting. Noninoculated seed was sown in the even-numbered rows and seed inoculated with spores of race L–2 in the odd-numbered rows. The results of this test are presented in table 1.

High infection was obtained with both dwarf bunt and L–2 on both dates

TABLE 1.—*Effect of Tilletia foetida (race L–2) on the development of dwarf bunt in Utah Kanred wheat, Clarkston, Utah, 1941–42*

| Row No. | Inoculum on seed | Percentage of plants in the first planting infected with | | | Percentage of plants in the second planting infected with | | |
|---|---|---|---|---|---|---|---|
| | | Dwarf bunt | Race L–2 | Dwarf bunt and race L–2 | Dwarf bunt | Race L–2 | Dwarf bunt and race L–2 |
| 1 | L–2 | 25 | 53 | 3 | 19 | 61 | 3 |
| 2 | None | 78 | 0 | 0 | 62 | 0 | 0 |
| 3 | L–2 | 12 | 65 | 12 | 16 | 61 | 3 |
| 4 | None | 71 | 0 | 0 | 71 | 0 | 0 |
| 5 | L–2 | 23 | 63 | 0 | 11 | 72 | 1 |
| 6 | None | 75 | 0 | 0 | 70 | 0 | 0 |
| 7 | L–2 | 44 | 20 | 0 | 11 | 66 | 6 |
| 8 | None | 9 | 0 | 0 | 67 | 0 | 0 |
| 9 | L–2 | 9 | 70 | 7 | 10 | 71 | 3 |
| 10 | None | 42 | 0 | 0 | 70 | 0 | 0 |
| 11 | L–2 | 12 | 68 | 0 | 6 | 73 | 6 |
| 12 | None | 83 | 0 | 0 | 88 | 0 | 0 |
| 13 | L–2 | 11 | 71 | 11 | 4 | 75 | 0 |
| 14 | None | 72 | 0 | 0 | 48 | 0 | 0 |
| 15 | L–2 | 10 | 84 | 4 | 5 | 74 | 1 |
| 16 | None | 76 | 0 | 0 | 56 | 0 | 0 |
| 17 | L–2 | 12 | 77 | 6 | 3 | 79 | 0 |
| 18 | None | 75 | 0 | 0 | 16 | 0 | 0 |
| 19 | L–2 | 12 | 75 | 8 | 1 | 80 | 4 |
| 20 | None | 56 | 0 | 0 | 46 | 0 | 0 |
| 21 | L–2 | 20 | 58 | 9 | 0 | 75 | 2 |
| 22 | None | 26 | 0 | 0 | 16 | 0 | 0 |
| 23 | L–2 | 18 | 66 | 7 | 0 | 88 | 3 |
| 24 | None | 63 | 0 | 0 | 11 | 0 | 0 |
| Average | L–2 | 17 | 64 | 6 | 7 | 73 | 3 |
| | None | 61 | 0 | 0 | 51 | 0 | 0 |

of seeding. In the first planting, the rows sown with clean seed averaged 61 per cent dwarf bunt, while those sown with seed inoculated with L–2 averaged 17 per cent dwarf bunt and 64 per cent common bunt. This reduction of 44 per cent in the dwarf bunt was duplicated in the second planting, thus indicating a definite depressing influence of L–2 on the dwarf bunt. As shown in table 1, this depressing effect on dwarf bunt was consistent except for one reversal in rows 7 and 8 of the first planting.

In the rows with common bunt (L–2), a small percentage of the plants

had both common and dwarf bunt. In a few cases, both kinds were found in the same plant, the same spike, or even in the same bunt ball, as evidenced by differences in culm height, spore ball characters, and chlamydospore markings. The rarity with which this occurred is further evidence that dwarf bunt does not readily develop in plants infected with race L–2 of common bunt.

The first test in Montana was designed to determine whether some races of common bunt are more effective than others in suppressing dwarf bunt. Hybrid 128 was inoculated with 5 races to which it is susceptible. Oro was inoculated with 2 races to which it is susceptible and with one (T–10) to which it is resistant. Two rows of Hybrid 128 and one row of Oro were grown for checks and all rows were planted in duplicate in each of two fields. The results are summarized in table 2.

TABLE 2.—*Data showing the depressing effect of several races of Tilletia caries (races T–2, 10, and 16) and T. foetida (races L–1 and 8) on the development of dwarf bunt in winter wheat, Bozeman, Montana, 1943–44*

| Variety | Inoculum on seed | Percentage of heads with | |
|---|---|---|---|
| | | Dwarf bunt | Common bunt |
| Hybrid 128 | L–1 | 6 | 88 |
| | L–8 | 3 | 92 |
| | None | 18 | 0 |
| | T–10 | 3 | 92 |
| | T–16 | 3 | 92 |
| | None | 23 | 1 |
| | T–2 | 6 | 86 |
| Oro | L–8 | 0 | 65 |
| | None | 20 | 0 |
| | T–16 | 2 | 65 |
| | T–10 | 5 | 5 |

The incidence of dwarf bunt the year of this test was relatively low. However, a consistently lesser amount occurred in rows with common bunt, regardless of the race used for inoculum or the variety inoculated (Table 2). This was true even on Oro inoculated with race T–10, to which it is resistant. In this test the smut percentages were determined on the basis of total heads per row. Consequently, no observations were made on the simultaneous occurrence of the two kinds of smut in the same plant.

In the second test in Montana, the varieties Golden and Yogo were inoculated with races L–7 and L–8, respectively. Each inoculation was replicated 8 times and each replication had a check row. Plantings were made in the fall of 1944 on each of two farms in dwarf bunt-infested soil. Very little dwarf bunt developed and no data were taken. This test was repeated the following year and the results are presented in table 3.

There was a marked depressing influence of the common bunt on dwarf bunt in both Golden and Yogo. The eight noninoculated rows of Golden had an average of 57 per cent dwarf bunt and no common bunt, in contrast with an average of 14 per cent dwarf bunt and 70 per cent common bunt in

the inoculated rows. Similarly, the noninoculated rows of Yogo had an average of 59 per cent dwarf bunt and no common bunt in contrast with 9 per cent dwarf bunt and 68 per cent common bunt in the inoculated rows. The depressing influence of the common bunt races L–7 and L–8 on dwarf bunt was consistent in all replications with both varieties.

TABLE 3.—*Data showing the depressing effect of races 7 and 8 of Tilletia foetida on the development of dwarf bunt in winter wheat, Bozeman, Montana, 1945–46*

| Variety | Inoculum on seed | Total heads | Percentage of heads with | |
|---|---|---|---|---|
| | | | Dwarf bunt | Common bunt |
| | | *No.* | | |
| Golden | L–7 | 140 | 18 | 69 |
| | None | 252 | 47 | 0 |
| | L–7 | 124 | 14 | 68 |
| | None | 237 | 49 | 0 |
| | L–7 | 142 | 12 | 71 |
| | None | 238 | 55 | 0 |
| | L–7 | 175 | 15 | 67 |
| | None | 292 | 54 | 0 |
| | L–7 | 161 | 7 | 79 |
| | None | 307 | 67 | 0 |
| | L–7 | 182 | 16 | 69 |
| | None | 285 | 59 | 0 |
| | L–7 | 164 | 14 | 72 |
| | None | 272 | 65 | 0 |
| | L–7 | 171 | 13 | 62 |
| | None | 284 | 59 | 0 |
| Average | L–7 | 157 | 14 | 70 |
| | None | 271 | 57 | 0 |
| Yogo | L–8 | 220 | 9 | 64 |
| | None | 381 | 55 | 0 |
| | L–8 | 365 | 9 | 75 |
| | None | 435 | 70 | 0 |
| | L–8 | 300 | 11 | 67 |
| | None | 413 | 38 | 0 |
| | L–8 | 307 | 8 | 77 |
| | None | 410 | 69 | 0 |
| | L–8 | 304 | 6 | 79 |
| | None | 377 | 68 | 0 |
| | L–8 | 230 | 12 | 60 |
| | None | 301 | 53 | 0 |
| | L–8 | 400 | 8 | 47 |
| | None | 397 | 56 | 0 |
| | L–8 | 413 | 6 | 77 |
| | None | 328 | 66 | 0 |
| Average | L–8 | 317 | 9 | 68 |
| | None | 380 | 59 | 0 |

As reported previously, dwarf bunt stimulates tillering in wheat. There is further evidence of this in the record of number of heads in individual rows in table 3. That is, Golden averaged 157 heads in the rows inoculated with common bunt and 271 heads in those with dwarf bunt only. The difference was less striking in Yogo, with averages of 317 and 380 heads, respectively, for the rows inoculated with common bunt and those having

dwarf bunt only. Unexplainable is the fact that two of the noninoculated rows of Yogo had fewer heads than the adjacent inoculated rows. Otherwise there was a marked increase in the number of heads per row, directly attributable to the tiller-stimulating effect of dwarf bunt.

In the data presented there is ample evidence that common bunt has a depressing effect on dwarf bunt development. The nature of this depressant action is not known. By reason of close proximity to the embryo, the common bunt spores had first opportunity to infect. This did not, however, decrease the opportunity for dwarf bunt infection. Apparently, however, it made conditions for dwarf bunt infection less favorable, either by preventing entrance of the infection hyphae or by suppressing parasitic development and sporulation on the host. Under some conditions both kinds of bunt can develop in the same plant (Table 1). This occurred only occasionally, despite its numerous opportunities. Consequently it would seem safe to conclude that physiologic races of *Tilletia caries* and *T. foetida* that cause the common bunt exert a deterring influence on dwarf bunt in the same plant. The races of *T. caries* and *T. foetida* used in these experiments seemed to be about equally effective in inhibiting dwarf bunt.

## SUMMARY

Common bunt of wheat was found to have a depressing influence on the development of dwarf bunt. This influence was exercised by all races and on all varieties tested. The nature of this influence is not known.

Evidence is presented to substantiate previous reports that dwarf bunt stimulates tillering in the infected wheat plants.

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# VIRUS INHIBITION BY EXTRACTS OF SPINACH

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## INTRODUCTION

In the course of studies on viruses of cabbage (*Brassica oleracea* var. *capitata* L.), it was noted that the strain of *Turnip virus 1* designated as cabbage virus A was infectious on spinach (*Spinacia oleracea* L.) (13). While this virus was not recovered by mechanical means from infected spinach, it was transferred readily by aphids. These observations raised the question as to whether there was released into spinach juice upon extraction a substance which inhibited the infectivity of the virus.

Many chemicals have been shown to inhibit the infectivity of viruses in the juice extracted from infected plants. Space does not permit a review of the literature on this subject here. It is worthy of attention, however, that in 1925 Duggar and Armstrong (4) reported that the crude extract of pokeweed (*Phytolacca decandra* L.) markedly inhibited the infectivity of the ordinary tobacco mosaic virus (*Tobacco virus 1* Johnson). Doolittle and Walker (3) reported that they were unable to recover the ordinary cucumber mosaic virus (*Cucumber virus 1* Johnson) from infected pokeweed plants by mechanical means although they did so readily with aphids. Johnson (6) observed that the extract of *Phytolacca rigida* Small almost completely inhibited the tobacco mosaic virus even after aging *in vitro* for "long periods." Others have noted that extracts of pokeweed and of a number of other species are inhibitive to certain viruses (1, 2). Of special interest are the inhibitory effects of extracts of several species of the Chenopodiaceae. Robbins (12) and Jones (8), each failed to recover the virus of sugar-beet mosaic from infected plants by mechanical extraction of the juice. Rawlins and Tompkins (11), however, were able to transmit the virus when they used carborundum as an abrasive. Grant (5) demonstrated that although sugar beet (*Beta vulgaris* L.), Swiss chard (*B. vulgaris* var. *cicla* L.), and spinach (*Spinacia oleracea* L.) were susceptible to ordinary tobacco-mosaic virus, the extracts from healthy plants of the three species greatly reduced the infectivity of the virus in juice extracted from infected tobacco plants. This inhibitive property was reduced greatly by dilution as well as by heating of the plant extracts. Johnson and Grant (7) noted irregularity in amount of infection with spinach juice containing the tobacco ring-spot virus.

This investigation is concerned primarily with the nature of the inhibitive property of spinach extract upon the tobacco-mosaic virus and the cabbage A strain of the turnip-mosaic virus. The inhibitive property of spinach extract was also compared with that of beet and chard. The reactions of the two viruses to spinach extract were compared with those of tobacco

ringspot, latent potato ringspot, necrotic potato ringspot, and cucumber mosaic viruses.

## MATERIALS AND METHODS

Cultures of all viruses used were maintained in systemically infected host plants kept in insect-proof cages in the greenhouse. The tobacco-mosaic virus, supplied by James Johnson, was maintained on tobacco and concentration of the virus in extracted juice was determined by the local-lesion method on *Nicotiana glutinosa* L. The cabbage-mosaic virus, isolated from naturally infected cabbage, was maintained on Jersey Queen cabbage plants and virus concentration determined by the local-lesion method on tobacco. The cucumber-mosaic virus was taken from naturally infected spinach and was maintained on tobacco and its concentration in juice tested by the local-lesion method on *Amaranthus caudatus* L. The tobacco-ringspot virus, secured from B. M. Duggar, was maintained on tobacco and was tested by the local-lesion method on the same host. The common potato-ringspot virus, which is latent in varieties of potato commonly used in the United States, and a second strain described by Larson (10), which causes necrosis in the variety Chippewa, were secured from R. H. Larson. They were maintained on tobacco and tested by the local-lesion method on the same host. They are designated in this paper as latent potato-ringspot virus and necrotic potato-ringspot virus, respectively.

Healthy plants were grown in a virus-free greenhouse which was kept free of insects by frequent fumigation. Uniform, vigorous plants were selected for virus concentration tests. Leaf tissue of infected plants was macerated in a mortar or by means of a vegetable grinder and the crude infectious juice was extracted from the pulp by squeezing through two layers of sterile gauze or by means by a hydraulic press. The crude juice was centrifuged and the supernatant liquid used. Spinach extract was made from greenhouse-grown plants or from fresh market supply. It was usually extracted in the same manner as infectious juice of virus-infected plants. In some cases a large quantity of spinach extract was made by freezing leaf tissue and filtering the juice through an asbestos-celite filter cake. The extract was stored at 4° C. for future use.

To determine relative concentration of a given virus in a series of juices, host plants were arranged according to a chosen experimental design, labeled, and the surplus leaves and growing point of each plant removed at least one day prior to inoculation. The Latin square design was used whenever possible. Finely powdered carborundum was lightly and evenly dusted over the entire leaf surfaces. In all cases one half of each leaf was inoculated with the test juice and the opposite half with a standard control juice. Inoculation was carried out with sterile glass spatulas of uniform surface area. While the half-leaf was being supported on a folded pad of absorbent paper toweling in one hand, the glass spatula, in the other hand, was dipped once into the inoculum contained in an evaporating dish and

rubbed once, lightly, over the leaf to distribute the inoculum. This preliminary rubbing was followed by two more rubs from the midrib outward with uniform pressure and movement so as to avoid excessive leaf injury and carry any excess inoculum out onto the inoculum pad. A toweling pad was discarded whenever it became damp and always after the final half-leaf inoculation of every treatment. A given test preparation was inoculated to the left half of each leaf of the plants in the first replicate and on alternate sides of the leaves in succeeding replicates. After each test sample of juice was inoculated, the control juice was inoculated to each opposite half-leaf. Inoculated plants were incubated for suitable periods at temperatures most conducive to lesion formation by the respective viruses. When lesion development was sufficiently advanced, detached leaves were placed on a ruled window of an electrically lighted counting box and the number of lesions was recorded with the aid of a mechanical counter.

### INHIBITIVE EFFECT OF THE EXTRACTS OF SPINACH, BEET, AND SWISS CHARD

A mixture of equal amounts of spinach extract and the crude juice of cabbage infected with the cabbage-mosaic virus was thoroughly agitated

TABLE 1.—*Average number of lesions per half-leaf of test plants inoculated with untreated infectious juice of cabbage and tobacco and with juice of each treated with equal parts of spinach extract*

| Trial No. | Infectious cabbage juice | | Infectious tobacco juice | |
|---|---|---|---|---|
| | Treated | Untreated | Treated | Untreated |
| 1 | 0 | 35 | 0 | 34 |
| 2 | 0 | 56 | 0 | 11 |
| 3 | 0 | 44 | 3 | 107 |
| 4 | 0 | 31 | 0 | 37 |
| 5 | 0 | 50 | 0 | 25 |
| 6 | 0 | 42 | 1 | 57 |
| 7 | 0 | 14 | ..... | ..... |

for five minutes and inoculated to half-leaves of actively growing tobacco plants. As a control, the opposite half-leaves of each plant were inoculated with a mixture of equal amounts of distilled water and the infectious cabbage juice. Similarly, juice from tobacco plants infected with the tobacco-mosaic virus was diluted 1–10 with distilled water and a portion of this dilution was mixed with equal parts of spinach extract; the remainder was diluted further with equal parts of distilled water. After thorough agitation the mixtures were inoculated, respectively to half-leaves of *Nicotiana glutinosa*.

The average number of lesions per half-leaf from several trials with infectious juice of cabbage and of tobacco is given in table 1. It is clear that the infectivity of the cabbage-mosaic virus was completely inhibited by this treatment in each trial, while the tobacco-mosaic virus was completely inhibited in four trials and nearly so in two trials.

TABLE 2.—*The inhibitive properties of extracts from leaves of garden beet, sugar beet, and Swiss chard compared with spinach extract*

| Source of extract | Ave. no. of lesions per half-leaf inoculated with treated and untreated infectious juice of virus indicated | | | |
|---|---|---|---|---|
| | Tobacco mosaic | | Cabbage mosaic | |
| | Treated | Untreated | Treated | Untreated |
| Spinach | 2 | 69 | 0 | 81 |
| Garden beet | 1 | 95 | ..... | ..... |
| Sugar beet | 4 | 63 | 0 | 71 |
| Swiss chard | 6 | 82 | 0 | 70 |

The extracts of leaves of garden beet, sugar beet, and Swiss chard were compared with that of spinach in like manner on the tobacco and cabbage mosaic viruses. The results are given in table 2. It is evident that the extracts were equal to that of spinach in their inhibitive properties.

### INHIBITIVE EFFECT OF SPINACH EXTRACT ON SEVERAL VIRUSES

In order to determine whether the inhibitive effect of spinach extract was specific to the tobacco and cabbage mosaic viruses, the tests were extended to several other viruses. The virus concentration of treated and untreated juice of tobacco systemically infected with the tobacco-ringspot virus, the latent potato-ringspot virus, and the necrotic-ringspot virus, respectively, was determined in the usual way by the number of local lesions on half-leaves of tobacco. Similar preparations from tobacco infected with the cucumber-mosaic virus were inoculated to half-leaves of *Amaranthus caudatus*. These virus suspensions were compared with those containing tobacco-mosaic virus and cabbage-mosaic virus, respectively. Undiluted juices were mixed with equal parts of spinach extract except for those containing tobacco mosaic and necrotic potato-ringspot viruses, which were diluted with 10 parts of distilled water before mixing with spinach extract. The results are given in table 3. At the concentration used, spinach extract was about equally effective against all six viruses.

TABLE 3.—*Effect of spinach extract upon infectivity of several plant viruses*

| Virus | Ave. no. of lesions per half-leaf inoculated with untreated juice and juice mixed in equal parts with spinach extract | |
|---|---|---|
| | Treated juice | Untreated juice |
| Tobacco mosaic | 1 | 45 |
| Cabbage mosaic | 0 | 39 |
| Tobacco ringspot | 0 | 93 |
| Latent potato ringspot | 0 | 53 |
| Necrotic potato ringspot | 0 | 481 |
| Cucumber mosaic | 1 | 21 |

### RATE OF THE INHIBITIVE REACTION

Preliminary experiments indicated that the inhibition of virus infectivity was instantaneous and that no further reduction occurred on standing. In additional experiments, spinach extract diluted 1:5 with distilled water and infectious juice containing tobacco-mosaic virus diluted 1:10 were mixed in equal proportions. Ten-cc. aliquots of this mixture were stored in plugged test tubes at 20° C. and half-leaves of *Nicotiana glutinosa* were inoculated after definite periods of incubation. Average numbers of lesions per half-leaf are recorded in table 4. The results substantiate those of earlier experiments in that the inhibition did not increase on standing. Moreover, little or no reactivation of the virus occurred during the four-hour incubation period.

TABLE 4.—*Comparative inhibitive effects of spinach extract at various intervals after mixture with juice containing tobacco mosaic virus*

| Interval between mixing juice and extract and inoculation of test plants | Ave. no. of lesions per half-leaf inoculated with treated and untreated juice | |
|---|---|---|
| | Treated | Untreated |
| *Hours* | | |
| 0.0 | 5 | 95 |
| 0.5 | 11 | 134 |
| 1.0 | 13 | 154 |
| 2.0 | 16 | 152 |
| 4.0 | 16 | 196 |

### LONGEVITY OF THE INHIBITIVE PROPERTY OF SPINACH, BEET, AND CHARD EXTRACTS IN VITRO

Certain experiments were set up to study the longevity of the inhibitive property of spinach extract *in vitro*. Five-cc. aliquots of the extract stored at 20° C. in cotton-stoppered test tubes were removed at various intervals up to six weeks and mixed in equal proportions with freshly extracted cabbage juice containing the cabbage-mosaic virus. Half-leaves of tobacco were inoculated with treated juice and with controls consisting of infectious juice diluted with equal parts of distilled water. In all cases, the aged spinach extract completely inhibited virus infectivity. When leaves and stems of spinach were stored in manila bags at 4° C. for six months, after which period the leaf tissue was in many cases dry and somewhat decomposed, the extract still completely inhibited infectivity of the cabbage-mosaic virus. To determine whether any reduction in the inhibitory effect would result from more rapid oxidation, air was continuously bubbled through spinach extract in a 12-inch glass column approximately 6 mm. in diameter for 24 hours. The extract was still completely inhibitive toward the cabbage-mosaic virus. In another experiment spinach extract stored for approximately 15 months at room temperature showed no significant reduction in inhibitory property toward the tobacco-mosaic virus. Beet and chard extracts were equally inhibitive to the tobacco-mosaic virus after

storage for 15 months *in vitro*. It was evident that the inhibitive property was quite stable *in vitro*.

EFFECT OF DILUTION OF SPINACH EXTRACT UPON THE INHIBITIVE PROPERTY

To secure a more accurate evaluation of the degree of inhibition, dilutions of the spinach extract with distilled water were made previous to mix-

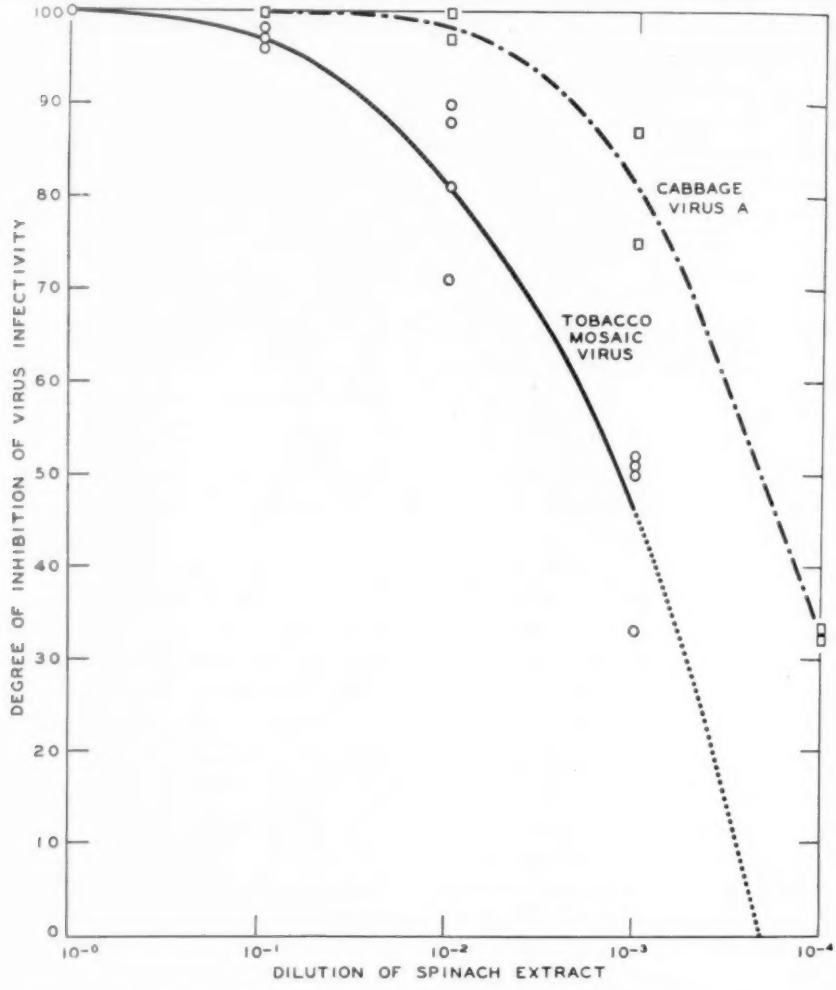


FIG. 1. The effect of dilution of spinach extract on inhibition of the cabbage-mosaic and tobacco-mosaic viruses. Degree of inhibition value was determined by the formula: $100 = \left(\frac{\text{number of lesions from treated juice}}{\text{number of lesions from untreated juice}} \times 100\right)$.

ture with infectious cabbage juice and with infectious tobacco juice. In each case the diluted extracts were mixed with equal amounts of infectious juice and the mixtures were inoculated to half-leaves of tobacco or *Nicotiana*

*glutinosa*. Corresponding half-leaves were inoculated with the respective juice diluted with equal amounts of distilled water.

The effect of dilution on the inhibitory property is shown in figure 1. The reduction in the inhibitory effect by dilution was somewhat more rapid in the case of the tobacco-mosaic virus than with the cabbage-mosaic virus.

Inasmuch as the tobacco-mosaic virus withstood a greater dilution than the inhibitor, an attempt was made to determine the permanency of the inhibitive effect. Infective juice containing the tobacco-mosaic virus was

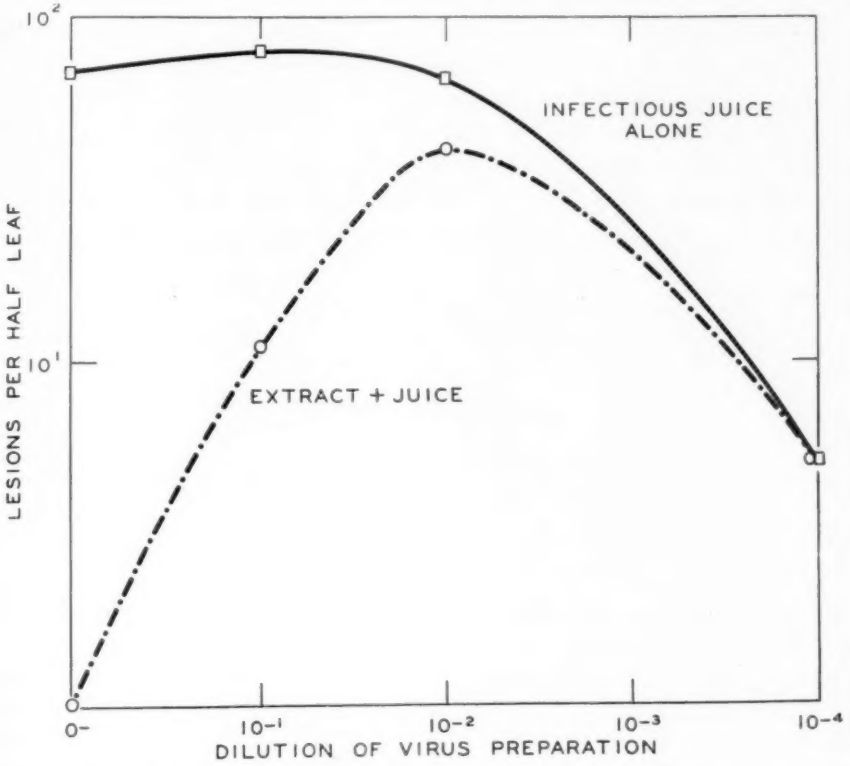


FIG. 2. Restoration of infectivity of the tobacco-mosaic virus by dilution of a mixture of infectious juice and spinach extract.

divided into two portions one of which was mixed with equal parts of spinach extract. This mixture was diluted with distilled water to $10^{-1}$, $10^{-2}$, and $10^{-4}$. The mixture and the various dilutions were each inoculated to six half-leaves of *Nicotiana glutinosa*. As a control, the second portion of the infectious juice was mixed with equal parts of water and then diluted further with water to $10^{-1}$, $10^{-2}$, and $10^{-4}$. These control dilutions were inoculated to the half-leaves opposite those inoculated with the corresponding treated dilutions. The average number of lesions per half-leaf is plotted in figure 2. It is shown that the infectious juice mixed with equal parts of water was highly infectious while the juice mixed with extract was

completely non-infectious. Dilution of the non-infectious mixture resulted in an increase in infectivity of the mixture up to dilution $10^{-2}$ at which point there appeared to be nearly complete dissociation of the virus and the inhibitive entity. Beyond that point the curve for the mixture followed closely that of the untreated tobacco juice and the reduction in infectivity of the former in dilutions above $10^{-2}$ was due primarily to the effect of dilution on the concentration of the virus.

### ADSORPTION OF THE INHIBITIVE FACTOR

Aliquots of spinach extract were mechanically stirred for five minutes with 10 per cent (by weight) Nuchar W, an activated charcoal. Following agitation, the charcoal-spinach extract mixture was filtered through an asbestos cake on Whatman No. 40 filter paper in a Büchner funnel. The filtrate was mixed in equal proportions with infectious cabbage juice. Another portion of the spinach extract was similarly treated with celite, a

TABLE 5.—*The effect of Nuchar W and celite as adsorptive agents on spinach extract*

| Virus | Ave. no. of lesions per half-leaf inoculated with infectious juice treated as indicated (T) and ave. no. of lesions on corresponding half-leaf inoculated with untreated juice (C) | | | | | |
|---|---|---|---|---|---|---|
| | Crude spinach extract | | Filtrate from spinach extract mixed with celite | | Filtrate from spinach extract mixed with Nuchar W | |
| | T | C | T | C | T | C |
| Cabbage mosaic | 0 | 46 | 0 | 60 | 0 | 93 |
| Tobacco mosaic | 0 | 341 | 10 | 280 | 251 | 253 |

siliceous adsorbent, and the filtrate likewise mixed in equal proportions with infectious cabbage juice. Controls consisted of equal parts of infectious cabbage juice and distilled water. Half-leaves of tobacco were inoculated. In a similar series of experiments, infectious tobacco juice was treated with the filtrates of the Nuchar W and celite-treated spinach extracts and inoculations were made to half-leaves of *Nicotiana glutinosa*. The results are given in table 5. Neither celite nor Nuchar W affected the inhibitive action of spinach extract on the cabbage-mosaic virus. Celite did not affect the inhibitive action of the spinach extract upon the tobacco-mosaic virus; Nuchar W, however, completely removed the factor inhibitive to the latter virus. This differential reaction indicated that perhaps two inhibitive factors were concerned in the spinach extract, one of which was inhibitive to tobacco mosaic and was adsorbed by Nuchar W. The inhibitory agent was not recovered from the charcoal filter cake by extraction with acid and alkaline solutions of various strengths.

The effect of certain other adsorptive agents on the inhibitory actions of spinach extract on virus activity was tested. Portions of spinach extract were agitated for five minutes with 10 per cent (by weight) of the respective

materials, and the mixtures were filtered through hard filter paper. The effect of the filtrates on the inhibition of virus infectivity was tested by mixing with equal amounts of tobacco-mosaic-virus extract. The ratio of the average number of local lesions per half-leaf produced by the filtrate-treated virus suspensions to that of the controls similarly diluted with water was as follows: Hy-flo Standard Super-cel, 1:100; wood charcoal, 1:100; celite, 4:100; animal charcoal, 6:100; Fuller's earth, 60:100; bone charcoal, 72:100; norite, 125:100; Nuchar W, 140:100. These results showed that Super-cel, wood charcoal, celite, and animal charcoal had very little effect on the inhibitory agent; Fuller's earth and bone charcoal apparently removed a part of the inhibitory agent; and norite and Nuchar W not only removed the inhibitory agent, but also increased the infectivity of the virus preparation.

Since the component of spinach extract which is inhibitive to the tobacco-mosaic virus could be removed by certain adsorptive agents, an experiment

TABLE 6.—*Restoration of tobacco-mosaic-virus infectivity by differential adsorption*

| Treatment | Average number of lesions per half-leaf inoculated with treatment preparation indicated (T) and untreated (C) | |
|---|---|---|
| | T | C |
| Infectious juice diluted 1:1 with water and then treated with norite | 172 | 159 |
| Infectious juice mixed with equal parts of spinach extract and then treated with norite | 176 | 182 |
| Spinach extract treated with norite and then mixed with equal parts of juice | 159 | 162 |

was designed to restore the infectivity to a noninfectious mixture by differential adsorption. Infectious tobacco juice was mixed in equal parts with spinach extract. Twenty cc. of this noninfectious mixture was thoroughly beaten for five minutes with two gm. of norite and the norite was then removed by filtration through hard filter paper. An aliquot of spinach extract alone was treated in a similar manner with norite and filtered previous to mixing with infectious juice. To allow for any effect of the norite on the tobacco-mosaic virus itself, infectious juice was diluted with equal parts of distilled water and the preparation likewise treated with norite and filtered. The filtrate or the filtrate mixed with infectious juice was inoculated to half-leaves of *Nicotiana glutinosa;* controls consisted of untreated infectious juice diluted 1:1 with distilled water. The results (Table 6) show that norite was effective not only in removing the inhibitor from spinach extract, but also in restoring the infectivity of a practically non-infectious mixture. When the adsorptive agent was applied to infective tobacco juice it increased the number of lesions over that of the control, suggesting that there might be a slight amount of an inhibitor in the tobacco juice. In a second experiment it was shown that practically the same

results were secured when the norite was filtered off before inoculation as when it was left in the mixture at the time of inoculation.

Norite treatment of a noninfectious mixture of spinach extract and cabbage-mosaic juice did not restore infectivity. This was in accord with a previous observation that adsorptive agents did not remove the substance inhibitive to the cabbage-mosaic virus. On the other hand norite readily removed the inhibitive effect of spinch extract on the necrotic potato-ring-spot virus. When a mixture of tobacco juice containing this virus and spinach extract was treated with this adsorptive agent more than 1000 lesions per half-leaf were produced by the filtrate, while none was produced by the untreated control mixture.

### EFFECT OF HEATING SPINACH EXTRACT UPON THE INHIBITIVE PROPERTY

In a study of the effect of heating spinach extract upon its inhibitive property the former was first filtered through an asbestos-celite cake to

TABLE 7.—*Effect of heat upon the inhibitive property of spinach extract*

| Treatment temperature (12 min.) °C. | Average no. of local lesions per half-leaf inoculated with preparations of heated spinach extract and infectious juice (T) and infectious juice alone (C), containing | | | |
|---|---|---|---|---|
| | Tobacco-mosaic virus | | Cabbage-mosaic virus | |
| | T | C | T | C |
| 55 | 0 | 80 | 0 | 21 |
| 60 | 0 | 112 | 0 | 14 |
| 65 | 80 | 101 | 0 | 38 |
| 70 | 103 | 101 | 0 | 20 |
| 80 | 111 | 114 | 0 | 15 |
| 90 | 85 | 75 | 0 | 12 |
| 100 | 101 | 95 | 0 | 24 |
| 125[a] | ..... | ..... | 0 | 75 |

[a] Treated for 15 minutes.

clarify the extract. Two-cc. aliquots of clarified spinach extract were drawn into thin-walled tubes, one end of each of which was sealed off. Three filled tubes were then inserted into a continually agitated water bath held within 0.1° C. of the desired temperature. After immersion for 12 minutes the tubes were cooled in running water, the drawn tips were broken off, and the contents of all these tubes forced into a 10-cc. centrifuge tube. The coagulum from the heat precipitation was thrown down in a centrifuge and the supernatant liquid was pipetted into another tube. The original reaction of the spinach extract, about pH 6.8, was not changed appreciably by the heat treatment. By this method a range of treatments at various temperatures was carried out.

The heated extracts were mixed in equal parts with tobacco juice containing the tobacco-mosaic virus and with cabbage juice containing the cabbage-mosaic virus, and half-leaves of the respective test plants were inoculated. Controls of the respective virus juices diluted with equal parts

of distilled water were inoculated to opposite half-leaves. Data from a representative series are given in table 7.

The two viruses did not respond to heated spinach extract in the same manner. The inhibitive property of the extract toward tobacco-mosaic

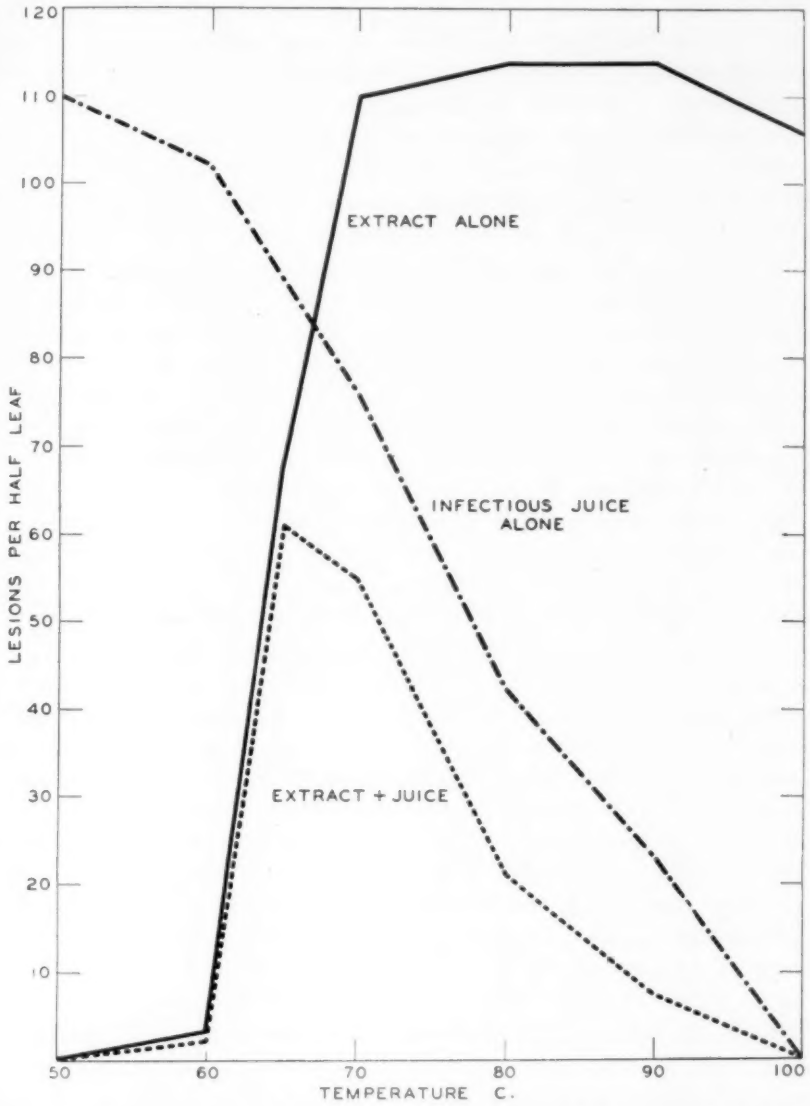


FIG. 3. The effect of various temperatures for 10-minute intervals on the infectivity of (1) a mixture of spinach extract and infectious tobacco-mosaic juice, (2) spinach extract alone, later mixed with infectious juice, (3) the infectious juice mixed with distilled water.

virus was thermolabile, being greatly reduced by exposure to 65° C. for 12 minutes and completely destroyed at 70°. On the other hand, heat did not destroy the inhibitive property of the extract toward the cabbage-mosaic

virus when exposed at 125° for 15 minutes. In most experiments in which the heat treatments were repeated similar results were obtained. In some cases the inhibitive effect of spinach extract toward the cabbage-mosaic virus was reduced by approximately one half when the former was heated at 100° for 15 minutes. The results indicated that either there were two distinct inhibitive substances in spinach extract, one thermostabile and one thermolabile, or that there was one substance which was destroyed in part by heat and in the resultant dilution of that substance the cabbage virus, being more sensitive than the tobacco virus, was completely inhibited.

In order to determine whether the infectivity of a noninfectious mixture of spinach extract and infectious tobacco juice could be restored by heating, spinach extract was mixed in equal proportions with infectious tobacco juice and 5-cc. aliquots of the mixture heated in test tubes immersed in a constant-temperature water bath for 10-minute intervals at various temperatures. Following treatment, the preparation was centrifuged and half-leaves of *Nicotiana glutinosa* were inoculated with the supernatant liquid. Two controls were employed. One control consisted of aliquots of spinach extract similarly heated and then mixed with the infectious juice. A second control consisted of the infectious juice mixed with equal parts of distilled water and similarly heated. The results of a representative experiment are presented graphically in figure 3. The spinach extract alone lost its inhibitive property completely at 70° C. With the infectious juice alone, number of lesions per half-leaf as a function of temperature followed a descending straight line curve. When the mixture of spinach extract and infectious juice was heated there was evidence of some dissociation of inhibitor and virus at 60°. At 65° where there was a discrepancy of about 30 lesions per half-leaf between the mixture and the infectious juice heated alone, the dissociation appeared to be nearly complete. Increase in temperature above 65° had the same effect on the virus in the mixture as it did on infectious juice alone. It was clear from these experiments that the infectivity of a mixture of infectious tobacco juice and spinach extract could be restored almost completely by heating to 65° C. Since infectious cabbage juice is inactivated at 56° to 58° for 10 minutes (13), it is obvious that this virus would be destroyed at a lower temperature than the inhibitor.

### EFFECT OF DIALYSIS UPON THE INHIBITIVE PROPERTY

Dialysis of celite-clarified spinach juice was carried out against frequently changed distilled water and flowing tap water by means of a modified Kunitz-Simms apparatus (9). Twenty-cc. aliquots of spinach juice were dialysed through a Visking sausage casing against four 300-cc. portions of distilled water and likewise against running tap water over a four-day period with the water cylinders mounted on a mechanical rocker. Both preparations contained in the sausage-casing bags were tested for inhibitory properties, as was the dialysed material after 1200 cc. of distilled water had been reconcentrated to the original volume by vacuum distillation at room

temperature. The three respective preparations were mixed in equal proportions with infectious tobacco juice containing the tobacco-mosaic virus and were inoculated to half-leaves of *Nicotiana glutinosa*. In another experiment, a similar dialysis procedure was carried out with a cellulose membrane. In this case, 25 cc. of spinach extract was dialysed against flowing tap water and also against a 150-cc. portion of distilled water over a 2-day period with only occasional agitation. The 150-cc. portion of distilled water was used without reconcentration. Inocula were prepared in the ratio of 1:1, using infectious cabbage juice, and inoculated to half-leaves of tobacco. The results of both experiments are given in table 8.

On the basis of the results secured with the dialysable portion of spinach extract on the infectivity of the cabbage virus, it was first thought that the

TABLE 8.—*The effect of dialysis upon the inhibitive factor in spinach extract*

| Membrane used in dialysis | Time in days | Virus | Average no. of lesions per half-leaf inoculated with juice treated with fraction indicated (T) and with untreated (C) juice | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Dialysis into distilled water | | | | Dialysis into tap water | |
| | | | Dialysed fraction | | Non-dialysed fraction | | Non-dialysed fraction | |
| | | | T | C | T | C | T | C |
| Sausage casing, Visking | 4 | Tobacco mosaic | 234 | 217 | 3 | 314 | 6 | 365 |
| Cellulose casing, Sargent | 2 | Cabbage mosaic | 9 | 80 | 0 | 90 | 0 | 90 |

inhibitive agent was slowly dialysable through the cellulose casing and that at the time the tests were made, an equilibrium had been established. Apparently enough inhibitive component had diffused outwardly to cause a 90 per cent reduction in infectivity despite the dilution effect of the distilled water, while yet enough inhibitive factor remained within the sac to completely inhibit infectivity.

The results secured with the standard sausage-casing membrane using tobacco-mosaic virus as an indicator of inhibition indicated that no dialysis of the inhibitive agent had taken place,—even when the outer distilled water containing the dialysed portion had been reconcentrated to the original volume. On the other hand, if the virus preparation had been diluted further before treatment some inhibitive effect might have been noticed; still, had any appreciable dialysis taken place, the lesion count would have far exceeded that of a mixture of infectious tobacco juice and untreated spinach extract which gave an average of four lesions per half-leaf. The factor inhibitive to tobacco-mosaic virus appeared, therefore, to be non-dialysable or very slowly so.

In accord with the differential thermostability of spinach extract reflected in the inhibition of infectivity of the tobacco-mosaic virus and the

cabbage-mosaic virus, a similar differentiating reaction was found in the fact that the factor inhibitive to the tobacco-mosaic virus did not dialyse while that inhibitive to the cabbage-mosaic virus alone did. This further indicated that two inhibitors might be present in spinach extract one of which was dialysable, while the other was nondialysable. On the other hand the results might be explained on basis of partial dialysis of the inhibitor and greater sensitivity of the cabbage-mosaic virus to it.

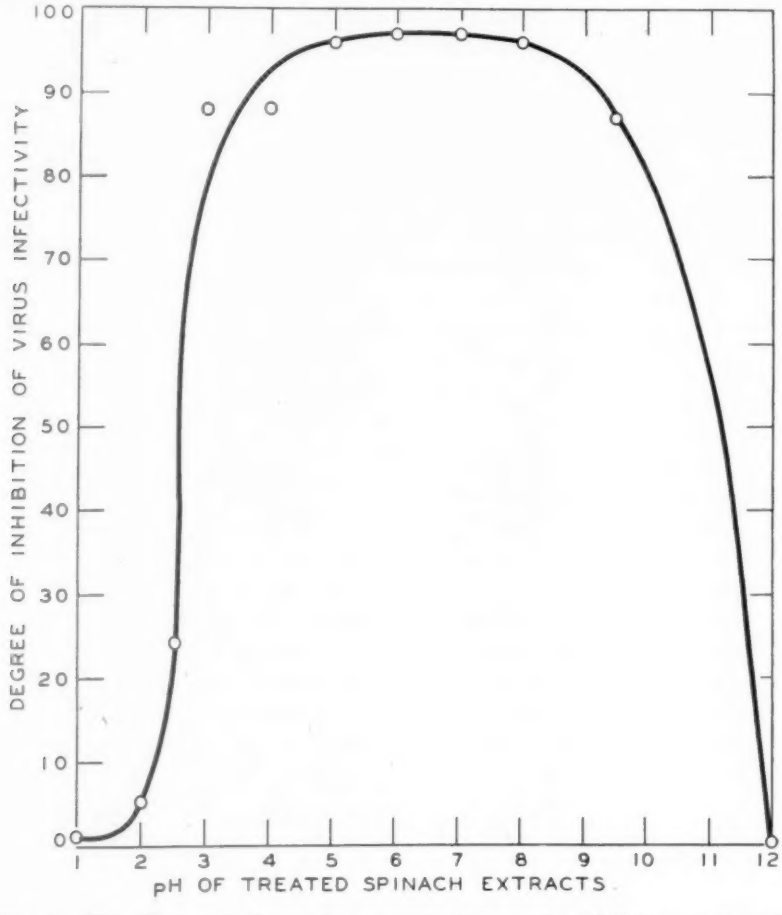


FIG. 4. The effect of hydrogen-ion concentration on the inhibition of virus infectivity by spinach extracts after readjustment to pH 7.

EFFECT OF HYDROGEN-ION CONCENTRATION ON THE INHIBITIVE PROPERTY

An experiment was designed to determine the effect of hydrogen-ion concentration on the inhibitory action of spinach extract. Adjustments in reaction of spinach extract ranging from pH 1 to 12 were made by adding the necessary amounts of N/1 HCl or N/1 NaOH to 4-cc. aliquots of spinach extract during constant agitation. Sufficient distilled water was added to the treated spinach extracts to equalize the different dilutions caused by the

addition of varying amounts of acid or base, as the case might be. Hydrogen-ion determinations were made by means of a Beckman pH meter during titration and immediately following a 24-hour treatment period at the desired pH. No preparation varied more than 0.2 of a pH unit during the treatment. All treated extracts were readjusted to pH 7, dilution effects again being equalized, and the inocula were prepared by mixing equal parts of the neutral extract with infectious tobacco juice containing the tobacco-mosaic virus. Inoculations were made immediately on half-leaves of *Nicotiana glutinosa*. A standard control consisting of equal parts of distilled water and crude infectious juice was inoculated on each opposite half-leaf. The degree of inhibition of virus infectivity by the treated extracts is shown in figure 4. Results indicated that the inhibitive factor was destroyed in extremely alkaline and extremely acid solutions. When the pH was changed to points within the range of pH 3 to 9.5 and readjusted to neutral there was no appreciable change in the inhibitory effect. No tests were made of the influence of the pH of spinach extract on the cabbage-mosaic virus.

### PRECIPITATION OF THE INHIBITORS

Spinach extract, infectious tobacco juice, and a mixture of the two were each precipitated by bringing them to 50 per cent ethyl alcohol through the addition of sufficient 95 per cent alcohol. The treated preparations were shaken thoroughly and allowed to stand 2½ hours. The precipitate in each case was thrown down in a centrifuge, the supernatant liquid pipetted off, and the residue washed twice with 50 per cent alcohol. The wash in each case was added to the original supernatant liquid which was twice reconcentrated to a syrupy consistency and finally restored to the original volume with water. The washed precipitate was resuspended in distilled water equal to the original volume. Each component of the treated spinach extract was then mixed with equal parts of tobacco-mosaic juice and the mixture inoculated to half-leaves of *Nicotiana glutinosa*, the control half-leaf in each case being inoculated with infectious tobacco-mosaic juice mixed with equal parts of distilled water. Inoculations of the other preparations were carried out in a similar manner. The results are summarized in table 9. The mixture of extract and juice was completely noninfectious. The precipitate from infectious juice when resuspended in water was nearly as infectious as untreated juice, while the supernatant liquid was also equal to the untreated juice in infectivity. This showed that part of the virus was precipitated and a considerable amount remained in the supernatant liquid. Apparently the original juice had the potentiality of producing many more lesions than could be measured on a single half-leaf. When the spinach extract alone was precipitated the inhibitor was completely removed from the supernatant liquid while it was not released from the precipitate when the latter was resuspended in the original volume of water. When the mixture of juice and extract was precipitated the infectivity of

the supernatant was partially restored and a portion of the virus particles carried down by the precipitate was released upon resuspension of the latter in water. It was shown by this experiment that the factor inhibitive to tobacco-mosaic virus was precipitated completely by 50 per cent alcohol. Moreover the infectivity of the noninfectious mixture of juice and extract was largely restored in the supernatant by precipitation with the same concentration of alcohol.

The supernatant liquid from precipitation of spinach extract with 50 per cent alcohol was tested against infectious cabbage juice. When juice and supernatant liquid were mixed in equal proportions the mixture was completely noninfectious while the cabbage juice control caused an average of 90 lesions per half-leaf. Thus it became clear that the tobacco-mosaic

TABLE 9.—*The effect of alcohol precipitation on tobacco-mosaic juice, and on the inhibitive property of spinach extract*

| Preparation of material | Ave. no. of lesions per half-leaf inoculated with treated (T) and untreated (C) juice | |
|---|---|---|
| | T | C |
| Spinach extract mixed with infectious juice | 0 | 147 |
| Precipitate of infectious juice resuspended in water | 131 | 189 |
| Supernatant liquid from precipitated infectious juice reconcentrated to original volume | 114 | 108 |
| Precipitate of spinach extract resuspended in water and mixed with equal parts of infectious juice | 154 | 148 |
| Supernatant liquid from precipitated spinach extract reconcentrated to original volume and mixed with equal parts of infectious juice | 148 | 153 |
| Precipitate of mixture of extract and juice, resuspended in water | 55 | 175 |
| Supernatant liquid from precipitated mixture of extract and juice, reconcentrated to original volume | 128 | 144 |

inhibitor could be separated from the cabbage-mosaic inhibitor by precipitation with 50 per cent alcohol.

The evidence already presented indicated that two inhibitive entities occurred in spinach extract, each of which, of course, might comprise one or more compounds. One of these entities was inhibitive to the tobacco-mosaic virus. Whether or not it was inhibitive to cabbage-mosaic virus was not ascertained. It was destroyed at 65° C. for 12 minutes; it was nondialysable; it was precipitated by 50 per cent alcohol. These characteristics suggested that the substance (or substances) in this component were noncrystalloidal, relatively high in molecular weight, and similar to proteins in some respects. The second component, on the other hand, was not inhibitory to tobacco-mosaic virus but decidedly so to the cabbage-mosaic virus. It was not destroyed at 125° C. for 15 minutes; it was dialysable; it was not precipitated by 50 per cent alcohol. These characteristics indicated that the substance (or substances) in this component was crystalloidal.

In view of the fact that free oxalic acid occurs to some extent in spinach

leaves as well as in those of beet and chard the effect of complete precipitation of such acid on the inhibitor was next tested. Spinach extract was first treated with norite and filtered to remove the tobacco-mosaic inhibitor. Another sample of extract was heated at 70° C. for 12 minutes and filtered to remove the tobacco-mosaic inhibitor. To 2-cc. aliquots of the respective filtrates three drops of 10 per cent calcium chloride solution were added and the heavy milky precipitate removed by filtration through an asbestos filter cake. The original and precipitated filtrates were each mixed in equal parts with cabbage-mosaic juice and inoculated to half-leaves. The opposite half-leaves were inoculated with a control consisting of the infectious cabbage juice mixed with equal parts of distilled water and treated with the same proportions of calcium-chloride solutions. The results are given in table 10. It is evident that the cabbage-mosaic inhibitor was completely or almost completely precipitated by calcium chloride.

TABLE 10.—*The effect of calcium chloride on the cabbage-mosaic inhibitor in spinach extract*

| Treatment of spinach extract before mixing with infectious cabbage juice | Total no. of lesions on 10 half-leaves inoculated with juice treated with the fraction indicated (T) and juice treated only with calcium chloride (C) | |
|---|---|---|
| | T | C |
| Treated with norite and filtered | 17 | 94 |
| Treated with norite, filtered, treated with calcium chloride and filtered | 70 | 68 |
| Heated at 70° C. 12 minutes and filtered | 0 | 99 |
| Heated at 70° C. 12 minutes, filtered, treated with calcium chloride and filtered | 74 | 86 |

## DISCUSSION

The foregoing experiments clearly demonstrate a striking inhibitory effect of crude spinach extract *in vitro* upon the infectivity of tobacco-mosaic and cabbage-mosaic viruses notwithstanding the fact that these viruses may infect spinach and increase in quantity in infected plants. Little is to be gained in speculation here concerning the physiological and biochemical processes involved in this difference between virus activity in living tissue and virus activity in the presence of extracted plant juice. In spite of the difficulty encountered in the mechanical transmission of the cabbage-mosaic virus from macerated host tissue, insect vectors readily extract the virus in an active form and transmit it to healthy plants. Nothing observed throughout this investigation indicates that the inhibitory action of certain plant extracts is due to modifications of the host plant inoculated with a given preparation. Rather it is indicated that distinct chemical substances present in the extracts of spinach and some other chenopodiaceous plants render the viruses noninfective. The inhibitive factor apparently remains stable and functional for a long time in non-sterile juice and it is not destroyed by the

usual fermentation processes. Its effect on the virus is immediate, but the infectivity of a noninfectious preparation can be restored by various treatments that separate the virus from the inhibitor.

It is suggested that two inhibitory substances are present in the spinach extract or that two fractions of the same substance occur. The first is thermolabile at 70° C., nondialysable, adsorbed by activated charcoal, unstable in extreme acid and alkaline solutions, precipitated by alcohol, and rendered inactive at dilutions of 1 : 10,000. The infectivity of a noninfectious preparation in which this inhibitor is operative can be restored by submitting the mixture to any of the above treaments. Such properties suggest a material colloidal in nature, of high molecular weight, and proteinaceous in character. The second inhibitory component of spinach extract as measured by its effect on the cabbage-mosaic virus is thermostable, dialysable, not precipitated by alcohol, and only slightly adsorbed by activated charcoals. Moreover its action is irreversible. It is probably crystalloidal and much simpler in chemical structure.

It is evident from this study that the common difficulty in transmitting viruses from infected chenopodiaceous hosts is due to an inhibition of virus infectivity by components of the plant extract. Inhibitors of varying potentialities have been reported to exist in other plants but usually in too low a concentration to be of any significance. On the other hand, these results demonstrate that the concentration of virus in crude infectious plant juice may not necessarily be accurately measured by the local-lesion method. Failure to recover viruses from inoculated plants by mechanical methods is certainly not to be taken as final negative evidence. Undoubtedly the so-called increase in virus concentration of a plant virus by treatment of the infectious extract is often due to the release of the virus from a virus-inhibitor complex.

## SUMMARY

The extracts of leaves of spinach, garden beet, sugar beet, and chard, when mixed in equal parts with the infectious juice of tobacco containing the tobacco-mosaic virus (*Tobacco virus 1*) and with the infectious juice of cabbage containing the cabbage-mosaic virus (*Turnip virus 1*), completely or almost completely inhibited the infectivity of those juices. When spinach extract was mixed in equal parts with plant juices containing the tobacco-ringspot virus, the latent potato-ringspot virus, the necrotic potato-ringspot virus, and the cucumber-mosaic virus, the infectivity of those juices was also completely or nearly completely inhibited. The action of the spinach extract was instantaneous and did not increase with time; the extract retained its inhibitive effect at least 15 months at room temperature.

The inhibitive effect upon the tobacco-mosaic and upon the cabbage-mosaic virus was reduced by dilution of the spinach extract. After treatment with norite and with Nuchar W the extract did not measurably inhibit the tobacco-mosaic virus but was still inhibitive to the cabbage-mosaic virus.

Heating of the extract to 70° C. for 10 minutes rendered it noninhibitive to the tobacco virus but when heated to 125° for 15 minutes it still completely inhibited the cabbage virus. The component inhibitive to the tobacco virus did not dialyse in sufficient amount to be measured but the dialysate was inhibitive to the cabbage virus.

When spinach extract was adjusted to various reactions from pH 1 to 12 for 24 hours and the reaction readjusted to pH 7 there was no appreciable change in the inhibitive effect on the tobacco-mosaic virus from pH 3 to pH 9.5; the inhibitive property was destroyed in extremely acid and extremely alkaline solutions.

Spinach extract was diluted with 95 per cent ethyl alcohol to the point where the mixture contained 50 per cent alcohol. After being shaken, allowed to stand 2½ hours, centrifuged, and washed with 50 per cent alcohol the supernatant liquid had no inhibitive effect on tobacco-mosaic virus; the precipitate, moreover, did not release any of the inhibitive material when resuspended in distilled water. When the supernatant liquid was mixed with cabbage-mosaic virus it was completely inhibitive.

When the tobacco-mosaic inhibitive factor was removed from spinach extract by treatment with norite or by heating the extract, and the norite or precipitate removed by filtration, the filtrates were still inhibitive to the cabbage-mosaic virus. When calcium-chloride solution was added to such filtrates and the precipitate was filtered off, the second filtrate was not inhibitive, indicating that the factor inhibitive to the cabbage-mosaic virus had been removed by precipitation with calcium chloride.

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# CLUBROOT OF TOBACCO: A WOUND-TUMORLIKE GRAFT-TRANSMITTED DISEASE[1]

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(Accepted for publication May 20, 1947)

In August, 1922, specimens of tobacco with enlarged roots were received from Campbell County, Kentucky. As these plants had some similarity to those attacked by root-knot nematode, they were carefully examined for nematodes, but none was found. Specimens were sent to Dr. N. A. Cobb on two occasions and both times he reported the disease was not caused by nematodes. It was given the name "clubroot" because of the similarity of the enlarged roots to clubroot of cabbage.[2]

The disease was observed later near Owensboro, where the root symptoms were associated with peculiar plant characteristics in half-grown plants. The lower leaves were normal in size and appearance but the upper leaves were abnormally short, giving the plant a pyramidal shape. The growth of the plant was stunted considerably.

In 1946, while plants were being examined for brown root rot, clubroot was found in Fayette, Owen, Boone, and Mason Counties. The diseased plants were usually growing poorly but could not easily be distinguished from other slow-growing but apparently unaffected plants except by examination of the roots.

Several plants were transplanted from the field to pots in the greenhouse for further study. One plant was given to Dr. L. M. Black for comparison with his wound-tumor disease which clubroot seemed to resemble closely.

Assuming that the disease was caused by a virus, some tests were made to determine whether the causal agent was systemic. Evidence that the causal agent was present in the above-ground parts of affected plants was obtained from a cutting of an affected plant and from grafting scions from affected plants on healthy tobacco plants. On October 10, 1946, a cutting was made from the top of a 2-foot-tall clubroot Burley tobacco plant. It was planted in a steam-sterilized mixture of sand and soil in a 4-inch pot and placed under a bell jar. On November 28 the cutting was removed and found to be fairly well rooted. The roots showed no signs of tumors, but a large tumor ¾ inch in diameter and several small tumors had formed over the cut surface of the cutting. All of the roots had developed from the large tumor. The cutting had grown very little, but the growing point curved toward the side on which the large tumor had developed. Following examination the cutting was planted in a ground bench in the greenhouse for further observation. On April 3, 1947, approximately 4 months after trans-

[1] The investigation reported in this paper is part of a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] Valleau, W. D., and E. M. Johnson. Tobacco diseases in Kentucky. Ky. Agr. Expt. Sta. Bull. 328. 1932.

planting and a few days after the plant had died, it was dug up for examination. There had been little if any growth of the top. There had been some development of the roots but it was largely confined to the production of new roots from the tumors and to the development of a few laterals from the larger roots. While the roots at the time of transplanting showed no tumors, they now had tumors at the points where lateral rootlets were pro-

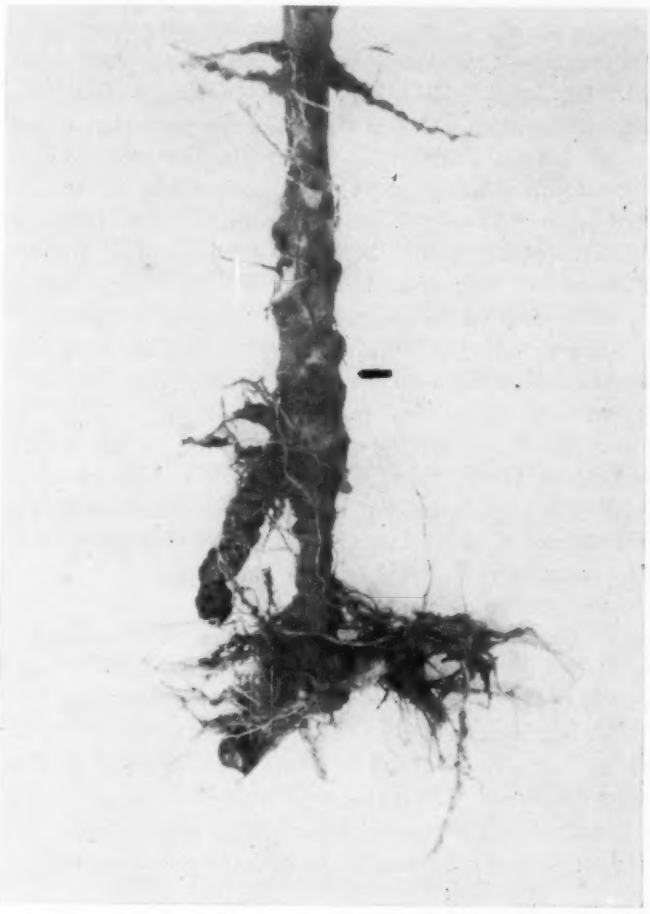

FIG. 1. Clubroot of Burley tobacco. A scion from a clubroot Burley tobacco plant was grafted on a healthy root of Ky 16 Burley. After union had taken place the plant was set in a ground bench with the graft union (arrow) 4 inches below the surface. Tumors may be seen on the scion roots. The stock root system is a mass of tumors.

duced. There was no indication that the large tumors on the base of the cuttings or the tumors on the roots had been caused by root knot nematodes.

Grafts were made on three Ky 16 Burley plants growing in 4-inch pots, using scions from three clubroot Burley plants. All made good unions, but when the roots were washed about a month after grafting they were found to have black root rot (*Thielaviopsis basicola*) and to have made but little root growth. There were no signs of tumors on the roots. One of the

plants, following washing and removal of many of the rootlets, was transplanted to sand and left for 5 weeks, when the roots were again washed. The new rootlets appeared normal but the roots from which they grew were irregularly swollen and similar in appearance to a mild natural case of clubroot. A second plant grafted October 18, 1946, was examined November 28, but no tumors were apparent. The plant was then set in a ground bench with the graft union 4 inches below the surface, in the hope that roots might develop from the scion. This plant sent out several shoots that bloomed at a height of about 2 feet and ripened seed. The leaves were small but otherwise normal. On April 4, 1947, 154 days after transplanting, the plant was dug and the roots washed. A few roots had developed from the scion, and these had small tumors at intervals where lateral rootlets developed. The Ky 16 root system, originally healthy, was made up of a few much enlarged and distorted roots definitely of the type found on naturally infected field plants (Fig. 1). Some of the younger roots had small tumors at points where rootlets originated, while the youngest roots appeared normal. It seemed that the tumors on the old roots gradually increased in size until the whole root appeared to be made up of an irregular mass of tumor tissue. A careful examination of the beadlike enlargements on the smaller roots indicated that root knot nematodes were not involved.

In the instance of the cutting and the two grafts the causal agent evidently was present in the growing points of the clubroot plants, because roots that developed from the cutting and from the scion had well-formed tumors. Furthermore, in the two grafted plants the causal agent appeared to have been transmitted from the scion to the previously healthy roots upon which they were grafted.

Because of the similarity of the tumors to those caused by Black's[3] wound-tumor virus and because both diseases appear to be caused by viruses, it is probable that the virus of clubroot is closely related to, if not the same species as, the wound-tumor virus. The virus of the wound-tumor disease is transmitted by the agallian leaf hoppers *Agallia constricta* Van Duzee, *Agallia quadripunctata* (Provancher), and *Agalliopsis novella* (Say). While Black did not test tobacco as one of the possible hosts of the virus, the wound-tumor virus has a very wide host range in which tobacco might be included.[4]

Since this paper was written the writer has seen an abstract of a paper by Trotter[5] in which he describes a disease of Kentucky and Burley tobacco growing in Italy which is very similar to club root. He attributed the tumors to *Bacterium tumefaciens.*

[3] Black, L. M. A virus tumor disease of plants. Amer. Jour. Bot. 32: 408–415. 1945.

[4] Since this was written Dr. Black has informed the writer that he has compared the symptoms of the clubroot virus and his wound-tumor virus in Turkish tobacco and while they are not identical they are very similar and may be caused by related viruses.

[5] Trotter, A. Sulla presenza di tumori radicali nelle coltivazioni di Tabacco di pieno campo. (On the presence of root tumors in tobacco plantations in the open field.) Ric. Ossvz. Divulg. fitopat. Campania ed Mezzogiorno (Portici) 10: 65–80. 1946. Abstr. in Rev. Appl. Mye. 26: 34. 1947.

# A NEW ANTHRACNOSE ON MELONS[1]

DESMOND DOLAN[2]

(Accepted for publication May 19, 1947)

## INTRODUCTION

While breeding muskmelons and watermelons at Spencerport, New York, in 1943, a severe and undescribed disease was found on both muskmelon and watermelon vines. Since 1943, it has become more prevalent, and in both 1944 and 1945 the melon crop of western New York was severely affected by this disease.

## SYMPTOMATOLOGY

The disease appears as small pink to light brown spots which coalesce to form long streaks on the petioles and vines. It seldom attacks the laminae of leaves and cotyledons. Diseased petioles become very brittle and break or turn brown and wither so that only the vine remains. The leaves wither and die mostly as a result of petiole infection, but occasionally streaks may be seen on the veins on the under side of the leaf. On the vines, the streaks may attain a length of 7 to 8 inches (Fig. 1). The terminal parts of such vines usually turn brown and die. In humid weather, the pink spore mass may be seen as a film on the disease lesions; but in dry weather, this character is absent.

## DESCRIPTION OF THE FUNGUS ISOLATED FROM DISEASED MELONS

Isolations from streaks on watermelon and muskmelon vines always yielded the same fungus. On potato-dextrose agar, the fungus produces a pink growth which spreads rapidly over the surface of the agar. The culture remains pink and does not turn black with age. The surface of the culture is smooth except for radial corrugations which are frequently most pronounced at the margin of the colony (Fig. 2). Pink spore slime is produced in great abundance. The spores are relatively small (8–10 $\mu \times 1.7$–3.3 $\mu$), 2-celled, and sub-rectangular.

In Petri-dish cultures from one to many spores are borne in heads on the ends of short conidiophores (Fig. 3, E and G). The head is not surrounded by a membrane. This was evidenced by the fact that (a) heads removed and placed in water disintegrated and the spores were immediately set free, (b) a very dense suspension of spores could be made by running water over the surface of the culture and (c) groups of spores could be seen lying on the surface of the agar as though they had been budded from a single conidiophore without sticking together to form a head (Fig. 3, F). Sometimes the

[1] This study was carried on while the author was a graduate student in the Department of Plant Breeding at Cornell University. The author wishes to express gratitude to Dr. W. H. Burkholder and Dr. E. M. Hildebrand for reading the manuscript and making helpful suggestions.

[2] Assistant Research Professor of Horticulture.

heads were spherical, possibly because of the presence of a film of water around the spore head.

Ten single-spore cultures were obtained by pouring a very dilute suspension of spores over the surface of solidified agar in a Petri dish, marking isolated single spores and removing them with a "biscuit cutter." Six of

FIG. 1. Muskmelon plants 3 days after inoculation with *Marssonina melonis* n. sp. Note streaks on stems, petioles and veins of cotyledons and leaves. Also note absence of spots in laminae of leaves and cotyledons.

the single-spore cultures were similar to the original culture, but three produced white growth on potato-dextrose agar. The spores of the nine pink isolates had the same dimensions as the spores of the original culture, but the spores of the white isolates were slightly smaller, measuring 5–7 $\mu \times 1.5$–3.0 $\mu$.

### PATHOGENICITY OF THE FUNGUS

An attempt was made to induce the disease artificially by inoculating six-week-old Bender muskmelon plants in the greenhouse in December with both the pink and white isolates. The plants were sprayed with a suspension of spores and then placed in a chamber with high humidity and a temperature of 75° F., but no infection resulted. Nongerminated spores were found in large numbers on the leaves 48 hours after inoculation. When 1 gm. of dextrose sugar was added to each 200 ml. of spore suspension before inoculation, abundant infection resulted and typical symptoms of the disease were produced. The disease symptoms developed on the petioles of the cotyledons 40 hours after inoculation. Sixty hours after inoculation, pink spots could be seen on the leaf petioles and on the vines, and these coalesced to form streaks that first became obvious 72 hours after inoculation. It was observed that petioles with pink streaks were very brittle and broke easily.

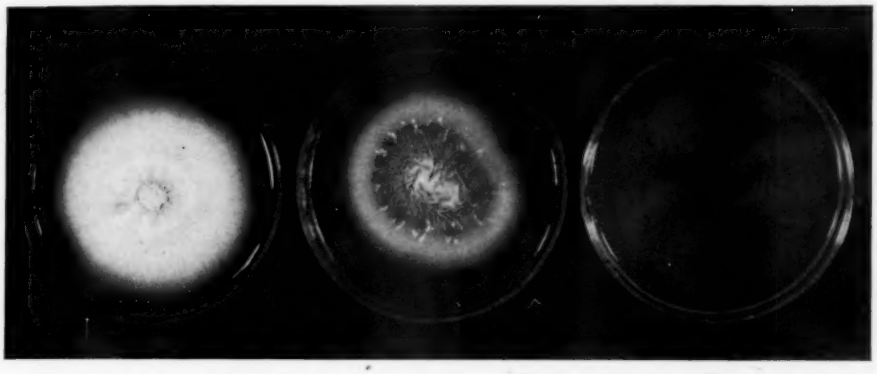

FIG. 2. Cultures of *Colletotrichum lagenarium* Ells. and Hals. (left), pink *Marssonina* (center) and white *Marssonina* (right) after 43 days on potato-dextrose agar at 48.2° F.

Later, the infected petioles collapsed and withered, producing a general plant appearance very similar to that seen in the field. The disease symptoms produced by the pink and white isolates were identical.

### HISTOLOGY OF THE DISEASE

The mycelium of *Marssonina* was intracellular (Fig. 3, A). This cross section of a disease streak shows a continuous fruiting layer from which the spores are produced in great abundance. No definite limits of the ascervuli were observed and it was concluded that when ascervuli are formed, they are probably gregarious, running together to form a continuous fruiting layer over the surface of the diseased tissue. At the border between the diseased and healthy tissue, the fruiting layer gradually merges into healthy tissue. No setae were observed protruding from the fruiting layer or at the line of demarcation between diseased and healthy tissue. The stroma is poorly developed and superficial, composed of loosely intertwined hyphae.

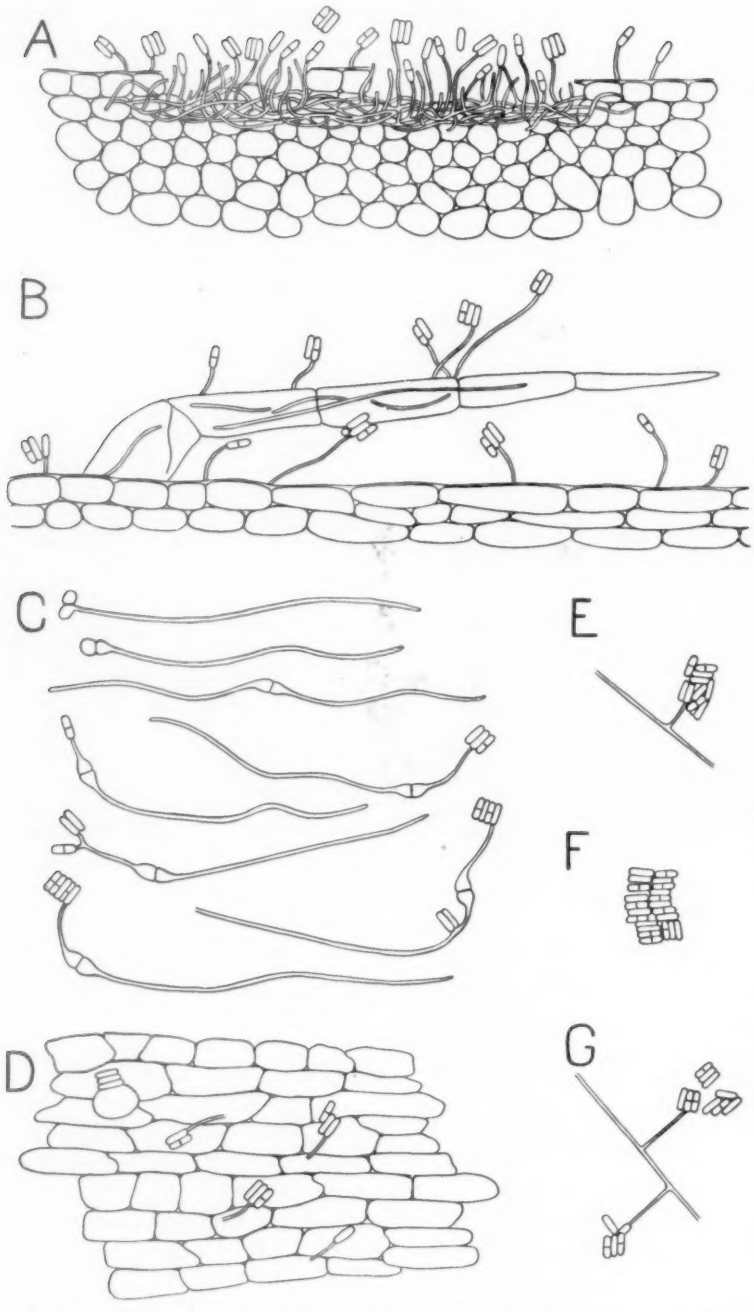


FIG. 3. A. Cross section of a streak lesion. B. Longitudinal section of streak lesion showing a trichome. C. Germinating spores of *Marssonina melonis* n. sp. D. Vertical aspect of a streak lesion showing conidiophores and spores on surface of leaf. E, F, and G. Spores being produced from conidiophores on the surface of potato-dextrose agar in a Petri dish. All 350 ×.

### TECHNICAL DESCRIPTION

**Marssonina melonis** n. sp.

Maculae parvae, rubicundulae vel pallide fuscae, coalescentes et lineas rubicundulas vel subfuscas vetustate atrofuscas formantes; ascervuli indefiniti, gregarii, struem fructificantem continuam, exiliter evolutam, superficialem, ex hyphis solute implectis compositam formantes; conidia subrectangulata, 8–10 $\mu \times 1.7$–3.3 $\mu$, medio 1-septata, protoplasmate hyalino.

Habitat: Parasitic on vines, petioles, and leaf veins of *Cucumis melo* var. *reticulatus* and *Citrullus vulgaris* in Munroe and Ontario Counties, New York.

Cultures and photographs have been deposited with the Department of Plant Pathology, Cornell University. Cultures were also sent to Professor David H. Linder, Herbarium of Cryptogamic Botany, Harvard University.

### PHYSIOLOGY OF MARSSONINA MELONIS

As mentioned previously, the difficulty in getting infection with *Marssonina melonis* during the winter in the greenhouse could be overcome by adding a small amount of sugar to the spore suspension before inoculation. Consequently, an experiment was planned to determine the effect of time of year and addition of sugar to the spore suspension on the amount of infection with two isolates (one pink and one white) of *Marssonina melonis;* and *Colletotrichum lagenarium* Ells. and Hals. was included as a standard for comparison.

Seeds of the Bender Surprise muskmelon were planted on four different dates: January 15, February 12, March 12, and April 16. The seeds were sprouted in paper towels, and 30 sprouted seeds were planted in each of 6 flats on the above dates. The plants were inoculated at the age of five weeks, two flats being inoculated with each of the three organisms. One of the two flats was sprayed with 200 ml. of a spore suspension to which no sugar had been added. The other flat was sprayed with 200 ml. of a spore suspension to which one gm. dextrose had been added.

The spore suspensions were prepared by washing spores from the surface of two-week-old Petri-dish cultures. The crude spore suspension was then centrifuged at 3000 r.p.m. for 5 min., the supernatant liquid was poured off and the original volume restored by adding distilled water. This was repeated three times until it was considered the spores were washed free of all nutrients that might have been carried over from the medium. After the final washing, the concentration of spores was adjusted to 100,000 per ml. which was equivalent to 50 spores per low power field using a 25× ocular.

After inoculation, the flats were placed in an infection chamber with high humidity and a temperature of 70–75° F. for 48 hours. Eight to ten days after inoculation, the degree of infection was measured according to a rating system: 0 meaning no infection, 1–3 meaning slight infection, 4–6 meaning medium infection, and 7–9 meaning severe infection. Each plant in the flat was rated and the 30 figures for each flat were averaged to give the degree of infection for each inoculation treatment.

The severity of infection for each organism for each date of planting, with and without the addition of sugar to the inoculum, is recorded in table 1.

Without the addition of sugar to the suspension of spores before inoculation the infection with both isolates of *Marssonina* was slight in the winter months when light conditions were poor. *Colletotrichum*, on the other hand, gave good infection in the winter months without the addition of sugar to the inoculum.

Examination of the leaves after inoculation revealed that lack of infection by the *Marssonina* isolates was due to nongermination of the spores.

TABLE 1.—*Average degree of infection with three organisms at different dates, when inoculated with and without the addition of sugar to the spore suspension before inoculation*

| Planting date | Inoculation date | Organism | Average degree of infection | |
|---|---|---|---|---|
| | | | Without dextrose | With dextrose |
| Jan. 15 | Feb. 19 | *Marssonina* (pink) | 0.0 | 7.3 |
| | | do (white) | 0.0 | 7.8 |
| | | *Colletotrichum* | 6.4 | 8.5 |
| Feb. 12 | Mar. 19 | *Marssonina* (pink) | 1.5 | 6.9 |
| | | do (white) | 1.8 | 7.7 |
| | | *Colletotrichum* | 5.9 | 8.7 |
| Mar. 12 | Apr. 16 | *Marssonina* (pink) | 6.5 | 6.6 |
| | | do (white) | 6.8 | 8.0 |
| | | *Colletotrichum* | 7.3 | 7.4 |
| Apr. 16 | May 21 | *Marssonina* (pink) | 7.8 | 8.1 |
| | | do (white) | 8.0 | 8.5 |
| | | *Colletotrichum* | 8.9 | 8.7 |

When there was no infection with *Marssonina*, nongerminated spores were still seen in abundance on the leaf 48 hours after inoculation.

### SPORE GERMINATION TESTS IN VARIOUS NUTRIENT MEDIA

Spore germination studies were carried on to determine (a) the minimum amount of dextrose needed for germination of *Marssonina* spores, (b) to determine if *Marssonina* spores would germinate without sugar in a mineral nutrient solution, (c) to determine if a higher percentage of *Marssonina* spores would germinate in a guttated fluid from melon plants growing in the light than from melon plants growing in the dark.

The germination tests were carried on in clean pyrex Petri dishes. Each Petri dish contained 10 ml. of solution made up as follows: 1 ml. of each nutrient under test, 5 ml. of spore suspension, and the remaining volume of redistilled water.

As a standard of comparison, spores of *Colletotrichum lagenarium* Ells. and Hals. were subjected to the same germination tests as those of the two *Marssonina* isolates. The suspension of spores of each organism was pre-

pared as follows: Spores were washed from the surface of two-week-old Petri-dish cultures. The suspension was then centrifuged at 3000 r.p.m. for 5 min., the supernatant liquid poured off, and the spores again suspended in redistilled water. The spores were given three such washings before being used in germination tests. After the final washing, the concentration of the suspension was adjusted to 200,000 spores per ml., which is equivalent to 100 spores per low power field using a 25× ocular. Five ml. of this suspension were placed in each Petri dish.

Germination counts were made on five low power fields using a 25× ocular at the end of a 12- to 24-hour period, and the average percentage of germination was recorded.

The average percentage germination of the three isolates in the various liquid media is recorded in table 2.

TABLE 2.—*Average percentage germination of spores of three organisms in various liquid media*

| Medium | *Marssonina* (pink) | *Marssonina* (white) | *Colletotrichum* |
|---|---|---|---|
| Redistilled water | 0.0 | 0.0 | 0.6 |
| Distilled water | 0.0 | 0.0 | 0.0 |
| Tap water | 0.0 | 0.0 | 3.5 |
| Dextrose in redistilled water | | | |
| 0.001 mg. per ml. | 0.0 | 0.0 | 0.8 |
| 0.005 do | 0.0 | 0.0 | 2.2 |
| 0.01 do | 0.0 | 0.0 | 4.5 |
| 0.05 do | 0.0 | 0.0 | 6.6 |
| 0.1 do | 3.2 | 2.0 | 9.1 |
| 0.5 do | 8.4 | 11.2 | 43.3 |
| 1.0 do | 39.5 | 29.9 | 93.1 |
| 5.0 do | 68.0 | 31.2 | 95.2 |
| 10.0 do | 72.6 | 65.1 | 95.4 |
| $KNO_3$ 0.505 gms. per ml. (5 mlmol./liter) $KH_2PO_4$ 0.372 gms. per ml. (2 mlmol./liter) $MgSO_4$ 0.120 gms. per ml. (1 mlmol./liter) in redistilled water | 0.0 | 0.0 | 0.0 |
| Above 5 mg. dextrose per ml. in redistilled water | 96.3 | 97.5 | 98.0 |
| Guttation from melon leaves in light | 63.8 | 71.2 | 96.6 |
| Guttation from melon leaves in dark | 0.0 | 0.0 | 2.2 |

These results show that the percentage germination of spores of both *Marssonina* and *Colletotrichum* increases as the sugar concentration is increased within certain limits. However, spores of *Colletotrichum* germinated at a lower sugar concentration than those of *Marssonina*.

A dextrose concentration of 5.0 mg. per ml. is approximately the threshold of germination for *Marssonina* spores. Melon plants growing in abundant light secrete an energy source in the guttation fluid which is apparently absent in guttated fluid from plants growing in the dark. Artificial infection is unsuccessful unless the spores are supplied with an energy source either naturally from guttation water or artificially by adding sugar to the spore suspension before inoculation. To insure infection of melon plants

growing in the greenhouse in winter with deficient light, it is necessary to add 5.0 mg. of dextrose to each ml. of spore suspension before inoculation.

### THE MANNER OF SPORE GERMINATION

A study of the manner of spore germination was undertaken as follows: Sterile pyrex Petri dishes with moist filter paper in the bottom were used as moist chambers for studying the germination of spores on microscope slides which had been previously dipped in 0.5 per cent dextrose solution.

Drawings of spore germination were made as shown in figure 3, C. It was observed that at the time of germination, the spores were very plainly 2-celled. They germinated from one or both ends; more frequently from both ends. In some cases, a conidiophore arose at one end of the spore and a germ tube at the other. The conidiophore budded off conidia in rapid succession so that they lay side by side as shown in figure 3, C.

### EFFECT OF HUMIDITY ON SPORE GERMINATION

Studies were conducted to determine the effect of a range of relative humidities on germination of spores of three organisms: (Mp) the pink

TABLE 3.—*Percentage germination of spores of three organisms in five relative humidities at the end of 24 hours*

| Salt | Gm. in 200 ml. | Percentage relative humidity | Percentage germination at end of 24 hours | | |
|---|---|---|---|---|---|
| | | | Mp | Mw | C |
| No salt: $H_2O$ only | ..... | 100.0 | 100.0 | 100.0 | 100.0 |
| $Na_2HPO_4 \cdot 12H_2O$ | 40 | 95.0 | 100.0 | 100.0 | 83.1 |
| $K_2CrO_4$ | 120 | 88.0 | 18.4 | 27.3 | 0.0 |
| $NH_4Cl$ | 75 | 79.5 | 0.0 | 0.0 | 0.0 |
| $NaNO_2$ | 190 | 69.0 | 0.0 | 0.0 | 0.0 |

*Marssonina*, (Mw) the white *Marssonina*, and (C) *Colletotrichum lagenarium* Ells. and Hals.

Medium-size bell jars (diam. 7 inches) were used as humidity chambers. Each bell jar was placed over a Stender dish containing a saturated salt solution to maintain the desired humidity. At their bases, the bell jars were sealed to glass plates with vaseline so as to make them air tight. The salt solutions used and the humidities maintained in the different chambers are recorded in table 3.

All chambers were kept at room temperature. The spores were germinated on microscope slides supported on glass rods above salt solutions.

Two hundred ml. of washed spore suspension were prepared as described previously and adjusted to a concentration of 200,000 spores per ml. One gram of sugar was added. Five ml. of the sugar solution spore suspension thus prepared were placed in a small test tube. One ml. $KNO_3$ solution (50 millimols per liter), 1 ml. $KH_2PO_4$ (20 millimols per liter), 1 ml. $MgSO_4$ (10 millimols per liter) and 2 ml. of redistilled water were added.

Each of the three nutrient solution spore suspensions were then stirred and smeared with a wire loop on ten clean microscope slides. The slides were then dried in front of an electric fan and two of the slides were placed in each humidity chamber.

Counts of the percentage germination were made at the end of 24 hours (Table 3). Three low power fields were counted on each slide, and the average percentage germination recorded.

Spores of the two *Marssonina* isolates germinated at a lower humidity than spores of *Colletotrichum lagenarium* Ells. and Hals.

### EFFECT OF TEMPERATURE ON GROWTH

Experiments were conducted to determine the effect of temperature on the growth of the two isolates of *Marssonina* as compared to *Colletotrichum lagenarium* Ells. and Hals. Sterile potato-dextrose agar was poured into 36 sterile Petri dishes so as to give a uniform depth of medium, and the agar

TABLE 4.—*Diameters in millimeters of colonies of three organisms (Mp = pink Marssonina, Mw = white Marssonina, and C = Colletotrichum lagenarium Ells. and Hals.) at various time intervals at four temperatures*

| Age of culture (Days) | Temperature | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 48.2° F. | | | 59.0° F. | | | 67.1° F. | | | 75.2° F. | | |
| | Mp | Mw | C | Mp | Mw | C | Mp | Mw | C | Mp | Mw | C |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 17 | 4 | 28 | 30 | 29 |
| 6 | 0 | 0 | 0 | 3 | 5 | 0 | 15 | 18 | 7 | 31 | 40 | 35 |
| 9 | 6 | 4 | 0 | 18 | 20 | 5 | 24 | 36 | 18 | 52 | 53 | 48 |
| 12 | 9 | 9 | 2 | 25 | 27 | 15 | 40 | 47 | 36 | 73 | 76 | 66 |
| 15 | 12 | 13 | 4 | 31 | 32 | 25 | 64 | 66 | 58 | Discarded | | |
| 22 | 20 | 19 | 5 | 57 | 61 | 38 | Discarded | | | | | |
| 29 | 31 | 32 | 6 | Discarded | | | | | | | | |
| 36 | 39 | 43 | 7 | | | | | | | | | |
| 43 | 44 | 49 | 8 | | | | | | | | | |
| 49 | 51 | 68 | 10 | | | | | | | | | |

was allowed to solidify. Twelve Petri dishes were inoculated with each of the three organisms by placing a loop of spore suspension in the center of each plate. Three plates inoculated with each organism were then placed at each of the following temperatures: 48.2°, 59.0°, 67.1°, and 75.2° F.

The diameters of the colonies were measured in millimeters at intervals of 3 days for 15 days, and after that time, at intervals of one week. The average diameters of the three replicates of each isolate at each temperature are recorded in table 4.

At low temperatures, the *Marssonina* isolates grew much more rapidly than the *Colletotrichum* isolate. The white *Marssonina* grows slightly more rapidly than the pink *Marssonina* at low temperatures. At the higher temperatures, the *Marssonina* isolates grew only slightly more rapidly than the *Colletotrichum* isolate. All three organisms displayed greatly increased growth rate with rising temperature and this trend was maintained to 75° F., the highest temperature under test.

## TESTING MUSKMELONS AND WATERMELONS FOR RESISTANCE TO MARSSONINA (PINK ISOLATE) AND COLLETOTRICHUM LAGENARIUM ELLS. AND HALS.

At the time the study was undertaken, two forms of anthracnose were prevalent in New York State. One form, lately discovered and described in this paper, was caused by an undescribed species of *Marssonina* (Fig. 1). The other form was the regular anthracnose caused by *Colletotrichum lagenarium* Ells. and Hals. (Fig. 4).

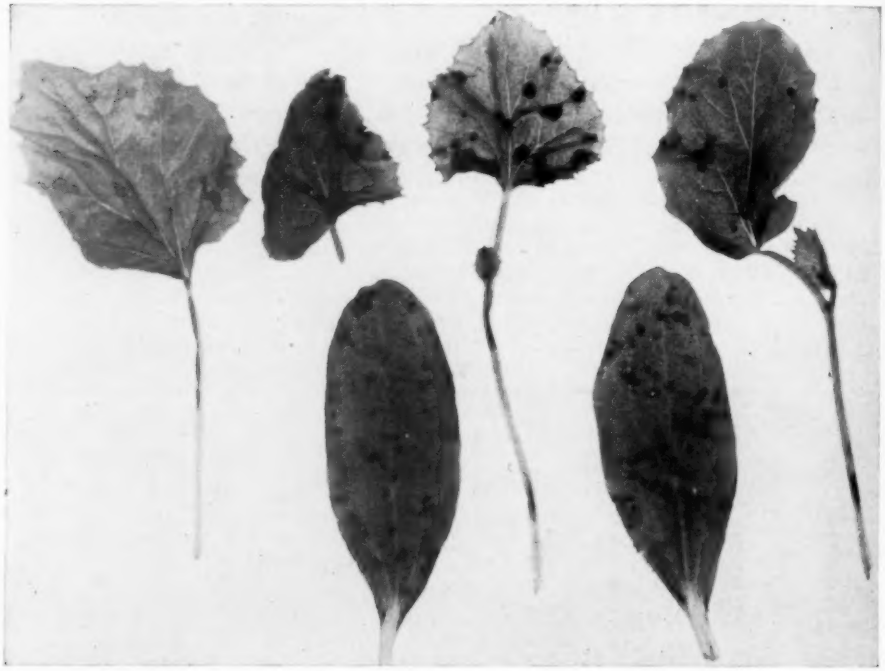

FIG. 4. Leaves, cotyledons, and stems of muskmelon plants 5 days after inoculation with *Colletotrichum lagenarium* Ells. and Hals.

In muskmelons, no resistance to either organism had been discovered. In watermelons, the McCrea resistant variety was reputed to be resistant to the regular anthracnose. With the hope of finding resistance to one or both of these diseases, muskmelons and watermelons from various sources were tested by artificial inoculation with both organisms in the greenhouse.

Forty seeds of each seed lot to be tested were germinated in moist paper towels in an incubator at 25° C. Eight sprouted seeds of each lot were planted in each of four 6-inch pots. One pot was allotted to each of four replicates in which arrangements were at random.

Twenty-five to thirty days after planting, two of the four replicates were inoculated with each of the two organisms, *Marssonina* (pink form) and *Colletotrichum*, by spraying with suspensions of spores of the organisms. The suspensions were prepared by washing spores from the surface of 2-week-

old Petri-dish cultures. The concentration of spores was adjusted to 50 per low power field using a 25× ocular. In order to insure infection with *Marssonina*, one gram of dextrose was added to each 200 ml. of spore suspension. After inoculation, the plants were placed in an infection chamber with high humidity; those inoculated with *Marssonina* for 40 hours and those inoculated with *Colletotrichum* for 48 hours.

TABLE 5.—*Average degree of infection of melons with Marssonina (pink isolate) and Colletotrichum lagenarium Ells. and Hals. after inoculation in the greenhouse*

| Variety | Source | Average degree of infection[a] | | | |
|---|---|---|---|---|---|
| | | *Marssonina* | | *Colletotrichum* | |
| | | Planting | | | |
| | | 1 | 2 | 1 | 2 |
| *Muskmelons* | | | | | |
| Conomon 43–192–n | Cornell University | 0.4 | 2.9 | 3.6 | 5.1 |
| Box No. 1—140666 | F.P.I.[b] | 1.2 | 4.9 | 6.8 | 7.6 |
| Box No. 1—140883 | do | 5.5 | 8.3 | 7.5 | 8.0 |
| Box No. 2—123493 | do | 2.0 | 5.2 | 6.1 | 7.0 |
| Box No. 3—125919 | do | 3.9 | 8.7 | 6.1 | 8.4 |
| Box No. 6—126114 | do | 6.5 | 7.7 | 7.8 | 8.2 |
| Box No. 8—125998 | do | 7.0 | 7.9 | 8.0 | 7.3 |
| Honeydew | Asgrow 37542 | 4.0 | 4.4 | 7.9 | 8.4 |
| Accession 106 | E. G. Anderson, 1945 | 4.1 | 4.5 | 6.4 | 7.5 |
| Honeydew | California, 1945 | 4.1 | 6.7 | 7.2 | 7.3 |
| Cassaba melon | Asgrow E 324.1 | 4.5 | 6.9 | 8.1 | 5.7 |
| Bender Surprise | G.L.F., Ithaca, N. Y. | 7.0 | 8.2 | 9.0 | 8.2 |
| White melon, *Cucumis melo conomon* | W. D. Enzie, Geneva, N. Y. | | 2.5 | | 5.9 |
| Freeman cucumber FC–2 | do | | 2.6 | | ..... |
| FC–3, Inbred 42–9 | do | | 2.4 | | 6.7 |
| Mardela 2–65 | do | | 4.1 | | 5.9 |
| Okase | do | | 7.9 | | 6.7 |
| FR 13–6, Inbred 42–11 | do | | 7.3 | | 6.7 |
| Aristogold 43–47 | do | | 6.7 | | 6.7 |
| $F_1$ Okase 5–39 × FC–2 | do | | 2.3 | | ..... |
| $F_1$ Aristogold 43–47 × FC–3 | do | | 3.0 | | 7.7 |
| $F_1$ K–3 × FR 13–6 | do | | 5.1 | | 7.8 |
| *Watermelons* | | | | | |
| McCrea resistant | Modesto, California | 8.1 | 7.9 | 2.9 | 5.6 |
| Honey Cream | Robson 345 | 2.9 | 3.6 | 7.8 | 8.5 |
| Tom Watson | Harris 43 | | 9.0 | | 9.0 |
| Dixie Queen | Harris 294 | | 8.3 | | 7.8 |
| Least difference required for significance | 19 : 1 | 1.8 | 1.8 | 2.5 | 2.1 |
| | 99 : 1 | 2.6 | 2.4 | 3.6 | 2.8 |

[a] 0 = no infection, 1 to 3 = slight infection, 4 to 6 = medium infection, 7 to 9 = severe infection.

[b] Federal Plant Introductions, obtained from W. D. Enzie, Geneva, N. Y.

Notes on degree of infection were taken 3–5 days after inoculation with *Marssonina* and 5–7 days after inoculation with *Colletotrichum*. Each plant in each pot was rated according to the degree of infection: 0 measuring no infection, 1–3 meaning slight infection, 4–6 meaning medium infection, and 7–9 meaning severe infection. The ratings of the plants in each pot were averaged, and the average degree of infection recorded. The average de-

grees of infection in the two replicates inoculated with each organism were again averaged and the least significant difference between means was determined.

Two different plantings were inoculated with each organism. The first planting was made on December 20. Two replicates were inoculated with *Colletotrichum* on January 15, and the final notes were taken on January 21. The other two replicates in the first planting were inoculated with *Marssonina* on January 17, and the final notes were taken on January 21. The

TABLE 6.—*Tabulation of frequency of occurrence of each infection rating in resistant and susceptible parents and in segregating backcross and $F_2$ progenies inoculated with Colletotrichum and Marssonina*

| | Frequency of occurrence of infection ratings | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. inc. | *Colletotrichum* | | | | | | | | | No. inc. | *Marssonina* (pink) | | | | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| *Resistant parents* | | | | | | | | | | | | | | | | | | | | |
| Conomon 43–192–n | 14 | | | 1 | 2 | 6 | 3 | 1 | 1 | | 16 | 2 | 6 | 7 | 1 | | | | | |
| White melon | 16 | | | | 1 | 0 | 5 | 9 | 1 | | 13 | | 10 | 3 | | | | | | |
| FC–3, Inbred 42–9 | 15 | | | | 3 | 2 | 1 | 6 | 2 | 1 | 16 | 3 | 5 | 7 | 1 | | | | | |
| *Susceptible parents* | | | | | | | | | | | | | | | | | | | | |
| Mardela 2–65 | 16 | | | 1 | 0 | 1 | 3 | 7 | 4 | | 14 | | 2 | 4 | 6 | 2 | | | | |
| F.R. 13–6, Inbred 42–11 | 16 | | | | | 2 | 0 | 3 | 5 | 6 | 14 | | | | | 4 | 2 | 7 | 1 | |
| Aristogold | 16 | | | | | | 4 | 8 | 2 | 2 | 16 | | | | 1 | 0 | 5 | 8 | 1 | 1 |
| *Backcross progenies* | | | | | | | | | | | | | | | | | | | | |
| 1. (W–2 × Mard. 2–65) × Mard. 2–65 | 36 | | | | | | 4 | 10 | 13 | 9 | 28 | 1 | 3 | 14 | 7 | 2 | 1 | | | |
| 2. (K–3 × F.R. 13–6) × F.R. 13–6 | 40 | | | | | | 7 | 15 | 13 | 5 | 40 | | 5 | 3 | 5 | 5 | 13 | 5 | 3 | 1 |
| 3. Aristogold 43–47 × FC–3 | 47 | | | | | 2 | 5 | 10 | 17 | 13 | 36 | | 6 | 0 | 10 | 7 | 8 | 4 | 1 | |
| *$F_2$ progenies* | | | | | | | | | | | | | | | | | | | | |
| 1. W–2 × Mard. 2–65 | 45 | | | | | | 12 | 19 | 9 | 5 | 31 | 4 | 11 | 5 | 8 | 3 | | | | |
| 2. K–3 × F.R. 13–6 | 52 | | | | 4 | | 6 | 12 | 17 | 13 | 36 | | 4 | 9 | 9 | 11 | 1 | 1 | 1 | |
| 3. Aristogold 43–47 × FC–3 | 41 | | | | | 1 | 6 | 16 | 11 | 7 | 46 | 6 | 14 | 11 | 7 | 1 | 1 | 3 | 2 | |
| 4. FC–2 × Arist. 43–5 | 42 | | | | | | 8 | 9 | 14 | 11 | 38 | 1 | 8 | 15 | 6 | 5 | 1 | 2 | | |
| 5. FC–2 × Mard. 46–7 | 44 | | | | | 2 | 9 | 13 | 15 | 5 | 45 | 1 | 7 | 10 | 14 | 8 | 2 | 2 | 1 | |

second planting was made on January 26. Two replicates were inoculated with *Colletotrichum* on February 20, and the final notes were taken on February 25. The other two replicates in the second planting were inoculated with *Marssonina* on February 22, and the final notes were taken on February 26.

Segregating backcross and $F_2$ progenies from crosses made by Professor W. Enzie at Geneva, New York, in which *Cucumis melo* var. *conomon* had been used as one of the parents, were included in the second planting. The individual plants in these progenies were rated according to degree of infection. The average degree of infection of each progeny was not determined. Instead, the ratings 1 to 9 were used as class values, and the frequencies of occurrence in each class were tabulated.

The average degree of infection with both organisms in both plantings is given in table 5. The frequency of occurrence of each infection rating

in the resistant and susceptible parents and in the segregating backcross and $F_2$ progenies is recorded in table 6.

In the first planting, muskmelons under test varied in resistance to *Marssonina* from the rather resistant Conomon to the very susceptible Bender, and many of the differences are significant. Two muskmelons from the Geneva collection, namely, 140666 and 123493, were significantly more resistant than other muskmelons tested. Honeydew melons and Accession 106 were significantly more resistant than four muskmelons from the Geneva collection and Bender. The Honey Cream watermelon was very resistant to *Marssonina*, but the McCrea resistant watermelon was very susceptible.

With regards to *Colletotrichum*, the McCrea resistant watermelon was very significantly more resistant than any other muskmelon or watermelon tested. Otherwise, the melons under test did not differ significantly in resistance to *Colletotrichum*.

In the second planting, infection with *Marssonina* was more severe than in the first planting. The Conomon melon, White melon, Freeman cucumber, and all their $F_1$ progenies, except one, were significantly more resistant than 140666, 123493, and most of the other muskmelons tested. White melon, Freeman cucumber, and one of the $F_1$ progenies were significantly more resistant than Asgrow's honeydew and Mardela, but Conomon 43–192–n and two of the $F_1$ progenies were not. Mardela, Asgrow's honeydew, Accession 106, 140666, and 123493 were significantly more resistant than Bender and most of the other muskmelons tested.

The Honey Cream watermelon was very significantly more resistant to *Marssonina* than other watermelons tested. The McCrea resistant watermelon was not significantly more resistant to *Marssonina* than the susceptible commercial varieties.

In table 6, it can be seen that backcross progeny 1 gave a higher proportion of plants resistant to *Marssonina* than backcross progenies 2 and 3. All three progenies had some resistant and some susceptible plants, but no ratios of resistant to susceptible plants are discernible. The $F_2$ progenies had a lower proportion of susceptible plants than the backcross progenies. However, all classes from high resistance to high susceptibility were represented. Such dispersion in a small population made fitting of the tabulated results to any genetic ratios impossible.

With regards to *Colletotrichum*, Conomon, Cassaba melon, White melon, and McCrea resistant watermelon were significantly more resistant than most of the other melons tested (Table 5). All plants of the segregating backcross and $F_2$ progenies had medium to severe infection with *Colletotrichum* (Table 6). Most of the plants in each progeny were severely affected. The proportion varied somewhat, but no definite resistance to *Colletotrichum* is discernible in the progenies.

## SUMMARY

A new anthracnose is described on muskmelons and watermelons. The most outstanding symptom is small pink to light brown spots coalescing with

age to form dark brown linear lesions. These streaks are mostly on the leaf petioles and vines. Diseased petioles become brittle and break or turn brown and wither so that only the vine remains. Frequently, the terminal parts of the vines become streaked and die.

The disease is caused by a species of *Marssonina*. Its most prominent features are its pink color in culture and its very abundant production of small, two-celled spores which cling together in groups.

During the winter months in a greenhouse with deficient light, the fungus is noninfectious when a spore suspension in distilled water is used as inoculum. Under such conditions, nongerminated spores are found in abundance on the leaves 48 hours after inoculation. Abundant infection results if one gram of dextrose is added to each 200 ml. of spore suspension before inoculation.

In germination tests, spores do not germinate at dextrose concentration less than 0.1 mg. per ml., and 5.0 mg. per ml. is necessary for abundant germination of spores. Spores of *Marssonina melonis* do not germinate in a solution of mineral salts, which indicates that it is an energy source that is needed, but the highest percentage germination is obtained in a medium containing both dextrose and mineral nutrients. Guttated fluid from melon plants growing in abundant light supports the germination of *Marssonina* spores, but there is no germination of spores in guttated fluid from melon plants growing in the dark.

Germination from both ends of the spores is frequent, and spores germinating in nutrient medium at times produce a germ tube at one end and a conidiophore bearing spores at the other end.

Spores of the two *Marssonina* isolates germinate at a lower humidity than spores of *Colletotrichum lagenarium* Ells. and Hals.

At low temperatures, the *Marssonina* isolates grow much more rapidly than *Colletotrichum lagenarium* Ells. and Hals. as measured by the diameters of Petri-dish cultures in millimeters.

Tests for resistance to *Marssonina* revealed that the Conomon melon and its relatives, White melon and Freeman cucumber, display definite resistance, and this resistance is dominant in $F_1$ progenies. Asgrow's honeydew, Accession 106, and Mardela also have considerable resistance. Several muskmelons from the Division of Plant Exploration and Introduction of the U. S. Department of Agriculture have more resistance than the commercial variety Bender and many other muskmelons tested. The Honey Cream watermelon is much more resistant than other watermelons tested. The McCrea resistant watermelon has no higher degree of resistance than susceptible commercial varieties.

Tests for resistance to *Colletotrichum* revealed that Conomon melon, Casaba melon, and McCrea resistant watermelon are more resistant than other muskmelons and watermelons tested.

RHODE ISLAND STATE COLLEGE,
KINGSTON, RHODE ISLAND.

## PHYTOPATHOLOGICAL NOTE

*Fasciation in Russet Burbank Potatoes.*[1]—In 1944, while surveying Idaho potato cellars in connection with the Emergency Plant Disease Prevention Project, U. S. Department of Agriculture, the senior writer occasionally found tubers with an odd symptom that he named "stitched end." Similar tubers found by grading crews in several lots of potatoes were subsequently submitted for diagnosis. A number of tubers were collected in 1944 and grown in 1945 and 1946 in plots at the Aberdeen Branch Experiment Station. It was found that the tuber symptoms were perpetuated and that plants having flattened stems were produced.

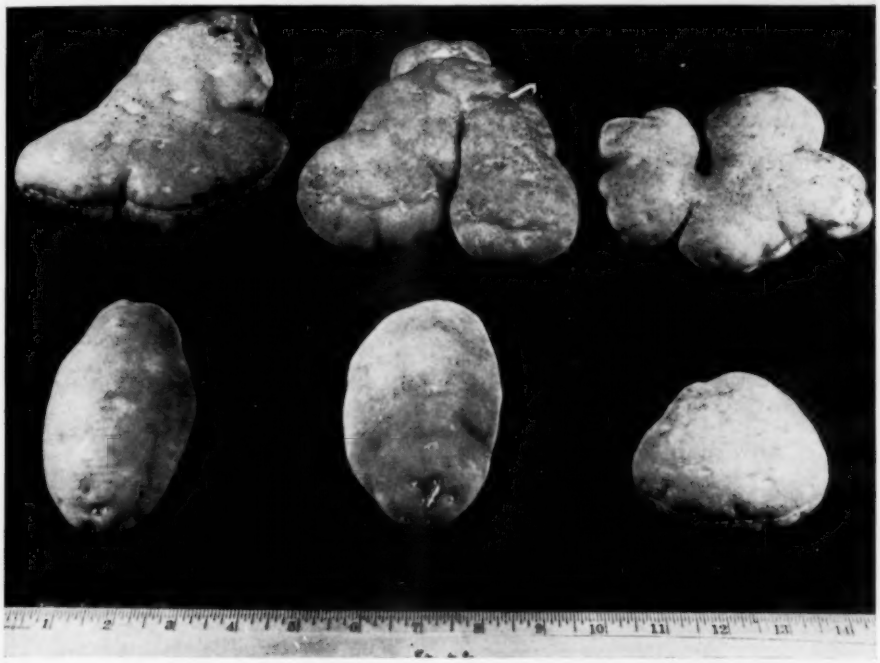

FIG. 1. Second generation fasciated tubers from the original collection made in 1944. Tuber at lower left, normal for the Russet Burbank variety. Remaining tubers illustrate various degrees of fasciation.

Affected tubers have a common symptom that is expressed to various degrees. This is the fusion of buds on the bud end (Fig. 1). At various intervals across the bud end are constrictions and ridges giving the appearance of a tightly sewed grain bag without ears. In nearly all specimens this symptom at once suggested the name "stitched end." Severely affected tubers tend to become flattened and wide on the bud end, giving a wedge-shaped tuber. The most severely affected tubers are flattened and have cleavages of various depths extending from the bud end toward the stem end,

[1] Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper No. 267.

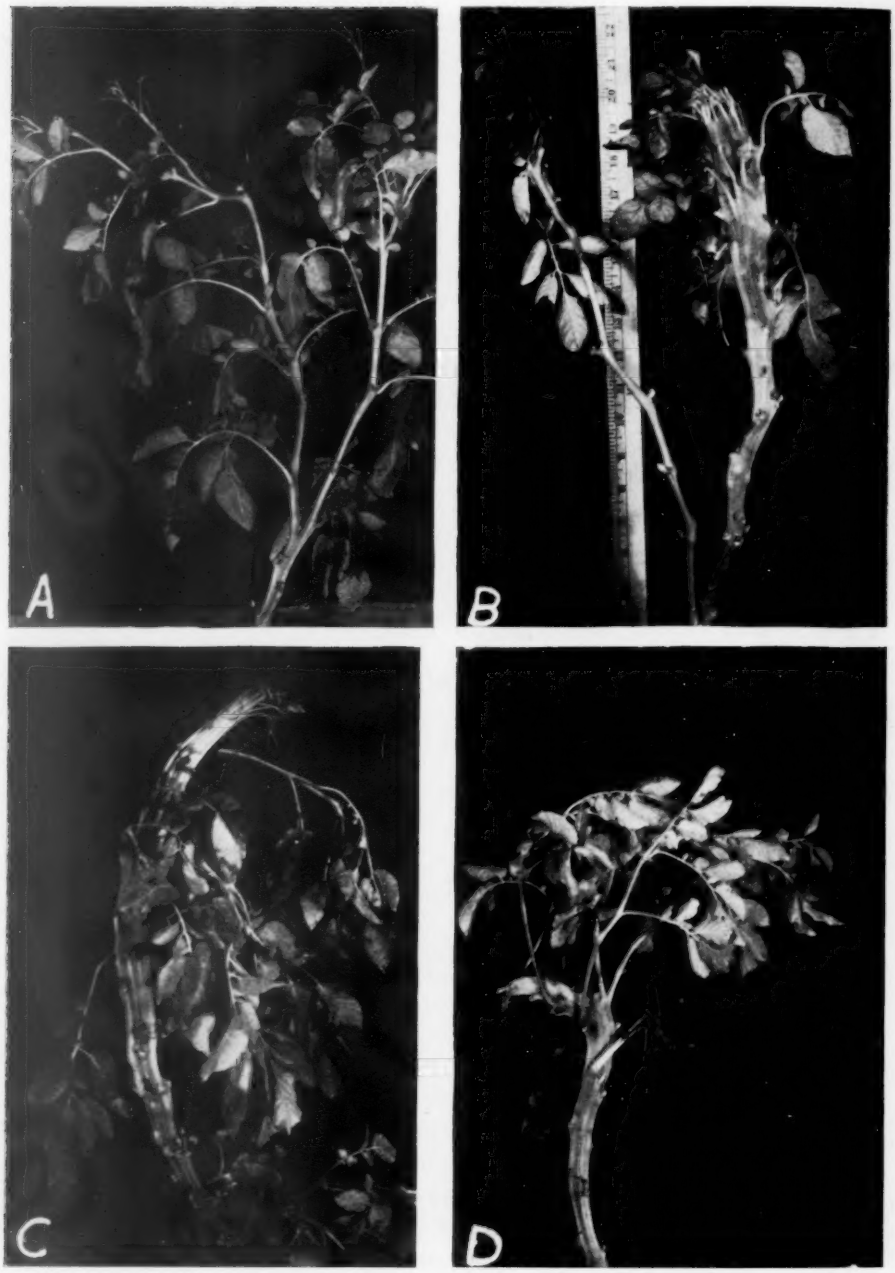


FIG. 2. Fasciation symptoms in the potato plant. A. Base of stem flattened. B, C, and D. Branching, leaf and bud arrangement, and fusion of inflorescences.

suggesting a fusion of tubers. In some cases, because of the extreme distortion, it is difficult to locate the stem end. All tubers in a hill are not equally affected and all gradations of tuber symptoms from apparently normal-shaped to the most severely distorted may be present. Eyes on the bud end of affected tubers appear to be small and shallow, and tend to sprout earlier than those on normal tubers.

Plants growing from the affected tubers may also be abnormal. Affected stems are flat or ribbon-like, but all stems from a hill are not fasciated (Fig. 2). Stems an inch or more wide may arise from the same seedpiece as normal-appearing stems. Intermediate stems may be oval shaped in cross section for only a portion of their length. Flattened stems are usually oval shaped at the soil level, but become wider and thinner toward the apex. The terminus of such stems may be curved downward describing some segment of a circle. Completely fasciated stems may be shorter than normal, although some such stems measured 29 inches above the soil surface.

Branching of fasciated stems also varies widely and with no regular pattern. Some completely fasciated stems are devoid of branches. The normal spiral arrangement of leaves and buds is lost, and several flattened nodes may be arranged as to give a whorl-like arrangement of leaves. Fasciated stems have been observed to branch in a dichotomous manner, producing two flattened, nearly equal, stems. In other cases, flattened stems branch to produce one or more laterals which may be either normal or flattened. Often, lateral branches are numerous on the distal half of fasciated stems, many of the branches growing to a greater height than the main axis. Lateral branches frequently are joined to the fasciated axis at an acute angle, and grow nearly parallel to the main axis. Leaves subtending lateral branches are sometimes missing and frequently out of natural position. Petioles may also show evidence of fasciation.

Inflorescences are frequently modified. In extreme cases, all peduncles are fused laterally, leaving only the pedicels and flowers free. When the flowers have abscissed, the free pedicels resemble somewhat the hairs of a brush. This extreme modification of the inflorescence is usually associated with the ribbon-type stem. Normal-appearing branches arising from fasciated stems frequently produce normal inflorescences in contrast to the fused type of the main axis. Flower stalk fusion may involve all stalks of the inflorescence or only two of them. The amount of fusion apparently reflects the extent to which the plant axis is fasciated.

The fasciated condition in potato tubers and plants, so far as known by the writers, is not described in literature. Although the exact nature of this fasciation or its causal factor is not known, it appears to be due to some genetic disturbance that is perpetuated in affected stock. Fasciation in several plant families has been described and attributed to gene mutations or various environmental stimuli.[2] The retention of plant vigor and tuber development by successive tuber generations suggests that this fasciation

[2] White, Orland E. The biology of fasciation and its relation to abnormal growth. Jour. Heredity. **36**: 11–22. 1945.

of potatoes is not due to a virus of the degenerative type. However, there is observational evidence that spindle tuber may mask the symptom of fasciation.

Affected plants and tubers are rare. The junior writer has found six fasciated plants in commercial fields during the past two years. It would be difficult or impossible to eliminate all affected tubers from a seed stock, as many tubers carrying the factor do not have symptoms, and seedpieces carrying the factor may not produce fasciated plants.—EARLE C. BLODGETT, formerly Plant Pathologist, Emergency Plant Disease Prevention Project, U. S. Department of Agriculture, Moscow, Idaho, and L. W. NIELSEN, Associate Horticulturist, Idaho Agricultural Experiment Station, Aberdeen, Idaho.

## ANNOUNCEMENT

The thirty-ninth annual meeting of The American Phytopathological Society will be held with A. A. A. S. at the Hotel Stevens in Chicago, Illinois, December 28–31, 1947. There will be joint sessions with The Potato Association of America, The Botanical Society of America, and The Mycological Society of America. All meetings will be held in Hotel Stevens.

Abstracts of papers to be presented at the meeting must be in the Office of the Secretary of The American Phytopathological Society by October 15, 1947.